
SECOND EDITION

Nutritional Assessment of Athletes

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Edited by
Judy A. Driskell
Ira Wolinsky



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Dedication

We would like to dedicate this book to Sylvia Wood, Project Editor at CRC Press, Taylor & Francis Group LLC. Sylvia worked with us and the chapter contributors for almost two decades. She made sure that the books and each of the associated chapters were correctly formatted, grammatically correct, appropriately referenced, and copyrights obtained if needed. She was hard-working, responsible, and easy to work with. She played a major role in helping us and the chapter contributors publish quality books and chapters. On January 26, 2010 she lost her years-long struggle with cancer. We miss her and her dedicated service.

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Preface

Through our books and researches, we are pleased to have played a small part in the recent, and rapid, growth of the science of sports nutrition, a phenomenon that continues unabated. Taken together, our series of monographs, edited volumes, and textbooks form an exhaustive and comprehensive corpus on the subject of sports nutrition, including assessment. These books have been very well received and we are proud. You have in your hands the latest book on the subject, the second edition of *Nutritional Assessment of Athletes*. Since the first edition, there have been important advances in critical areas of nutritional assessment and these are included. In-depth discussions of important topics of interest to health and nutrition professionals as well as the motivated layman and the weekend athlete are presented. As before, the volume covers a wide span of nutritional assessment and brings you the latest authoritative information from experts. As such, it may be used as a resource and a textbook.

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Dr. Driskell is a member of numerous professional organizations, including the American Society for Nutrition, the American College of Sports Medicine, the International Society of Sports Nutrition, the Institute of Food Technologists, and the American Dietetic Association. In 1993 she received the Professional Scientist Award of the Food Science and Human Nutrition Section of the Southern Association of Agricultural Scientists. In addition, she was the 1987 recipient of the Borden Award for Research in Applied Fundamental Knowledge of Human Nutrition. She is listed as an expert in B-complex vitamins by the Vitamin Nutrition Information Service.

Dr. Driskell co-edited the CRC book *Sports Nutrition: Minerals and Electrolytes* with Constance V. Kies. In addition, she authored the textbook *Sports Nutrition* and co-authored the advanced nutrition book *Nutrition: Chemistry and Biology*, both published by CRC. She co-edited *Sports Nutrition: Vitamins and Trace Elements*, first and second editions; *Macroelements, Water, and Electrolytes in Sports Nutrition*; *Energy-Yielding Macronutrients and Energy Metabolism in Sports Nutrition*; *Nutritional Applications in Exercise and Sport*; *Nutritional Assessment of Athletes*, first edition; *Nutritional Ergogenic Aids*; *Sports Nutrition: Energy Metabolism and Exercise*; *Nutritional Concerns in Recreation, Exercise, and Sport*; and the current book, *Nutritional Assessment of Athletes*, second edition, all with Ira Wolinsky. She also edited the books *Sports Nutrition: Fats and Proteins* and *Nutrition and Exercise Concerns of Middle Age*, published by CRC Press. She has published almost 200 refereed research articles and 20 book chapters as well as several publications intended for lay audiences and has given numerous presentations to professional and lay groups. Her current research interests center on vitamin metabolism and requirements, including the interrelationships between exercise and water-soluble vitamin requirements.

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Dr. Wolinsky is a member of the American Society for Nutrition, among other honorary and scientific organizations. He has contributed numerous nutrition research papers in the open literature. His major research interests relate to the nutrition of bone and calcium and trace elements and to sports nutrition. He has been the recipient of research grants from both public and private sources. He has been the recipient of several international research fellowships and consultantships to the former Soviet Union, Bulgaria, Hungary, and India. He merited a Fulbright Senior Scholar Fellowship to Greece in 1999.

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Section I

Dietary Assessment of Athletes

1 Estimation of Food and Nutrient Intakes of Athletes

*Robert J. Moffatt, Virginia B. Tomatis,
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1.1 INTRODUCTION

To ensure optimal performance it is vital to understand all aspects of an athlete’s preparation, including the diet. If a full understanding of the athlete’s nutritional status is to be learned, it is critical that this assessment be accurate and complete. This chapter examines the use of the dietary assessment as well as the proper analysis of dietary reports with a special emphasis on the athletic population.

There are various methods available to assess the nutritional status of an individual. It is often helpful to combine multiple methods to obtain a more comprehensive and accurate assessment. Sports dietitians should be aware of special considerations regarding the nutritional assessment of athletes, such as misreporting, snacking, fluid intake, and weight management. In addition, it is important that nutritional assessment be individualized according to the type of athlete and periodization (cycles) of training, as well as the location in which training and/or competition may take place.

Once the assessment has been obtained, dietary analysis should be performed to translate food intake into nutritional recommendations for individuals and populations. There are several options available to analyze food intake, such as an escalating variety of software programs and databases. Special considerations should be taken into account when choosing a food analysis method, such as the cost and output of the software as well as the quality of the nutritional database. New and ethnic foods, along with fluid and supplement analysis, are important components that should be included in the dietary analysis process.

In consideration of the issues stated above, this chapter addresses the following: (1) methods of assessing food intake, (2) special issues with assessing food intake in athletes, and (3) translation of dietary assessment into analysis.

1.2 METHODS OF ASSESSING FOOD INTAKE

The ability of the sports nutritionist to determine an athlete’s dietary intake and to consequently analyze his or her nutrient status is important. Reliable and accurate ways to assess food intake using food diaries, 24-hour dietary recalls, and food frequency questionnaires serve to assess food and nutrient intake in various ways.

The use of a specific method can be determined by the purpose of the assessment and other factors such as time and ability of the patient or athlete to record or recall specific intake.

Although these methods have the capability of being accurate and reliable when used properly, the dietitian must be aware of several issues that may arise and lead to decreased accuracy in analysis.

This section discusses the various methods of food intake and nutrient analysis.

1.2.1 DIET RECORDS

A diet record consists of all the food and beverages a person consumes in a certain amount of time. Three-day diet records are most often used (preferably two weekdays and one weekend day) to determine an individual's daily food and beverage consumption. Seven-day food diaries are more time consuming but may afford a more complete picture of the diet. It should be noted that diet records lasting an extended amount of time are not always as accurate as more concise diet records.¹ This is due to the fact that individuals may absent-mindedly forget to write down the information daily or may find the task tedious and redundant. The 7-day diet record is one of the most common approaches when assessing an individual's diet.¹ In general, the more information collected and the more details provided, the more accurate the conclusions.

After completion of the food diary it is important that the record be analyzed by a trained nutrition practitioner and preferably a registered dietitian. To ensure completeness and further accuracy regarding specific portion sizes, this is best conducted with the athlete.

When providing an individual with the task of assembling a diet record, a clear description of what items should be recorded as well as instruction on how to record the information should be provided. The individual should also be told what information will be drawn from the diet record. Reviewing specific tips on creating a diet record and the details that need to be present will assist the dietitian in subsequent visits and during analysis. As discussed later in this chapter, snacking and misreporting are issues that need to be considered when asking an individual to complete a diet record.

1.2.2 TWENTY-FOUR HOUR DIETARY RECALL

Twenty-four hour dietary recalls are often used as a quick nutrition assessment and many times can be used on an impromptu basis to determine an individual's daily intake. A dietitian will ask an individual to list the foods and beverages that he or she has consumed within the past 24 hours. When doing so, it can be advantageous to first review with the individual the past day's events, which then can be used to help recall specifics about dietary consumption.² The 24-hour dietary recall can be performed by two different methods. The first is when the dietitian asks the individual to start from the beginning of the previous day and provide in detail all of the food and beverages consumed from the beginning of the day before. The second method starts with the current day and works backward. For example, the individual

would be questioned on what he or she ate prior to this visit and then work back over the past 24 hours. Both methods allow the dietitian to use the individual's activities as a way to assist in recalling his or her dietary intake. A 24-hour dietary recall can take approximately 15 to 30 minutes to perform; however, it can take considerably longer if the individual has had mixed dishes or different foods.^{1,2} Both quantity and food preparation play a major role when performing a 24-hour dietary recall. An advantage of this method is that it can be done in person or over the telephone in a brief amount of time.

A main concern with a 24-hour dietary recall is its misrepresentation of the usual diet. It is important to ask the individual if the diet consumed within the last 24 hours is a normal diet or if it was a variation from the norm. As one might expect, the 24-hour recall is also very dependent on the individual's short-term memory.² A further complication in recalling food relates to its preparation, and accuracy is likely to be less if the diet was not prepared by the individual.

1.2.3 FOOD FREQUENCY QUESTIONNAIRES

Food frequency questionnaires can assist in determining, on average, the amount of a specific macro- or micronutrient an individual consumes. It too is highly dependent upon the individual's memory and ability to estimate the quantity of a particular food or food group. A list of foods is given to the individual and he or she is asked to determine how often each food was consumed during a specific period, usually ranging from one day to several months. A limitation of food frequency questionnaires is its specificity to certain populations.¹ Overreporting can be a major factor in this type of diet analysis.³ [Table 1.1](#) provides a brief description of the dietary analysis methods and their applications, along with advantages and disadvantages of each.

1.2.4 ISSUES WITH NUTRITION ASSESSMENT

A nutrition and dietary assessment is an integral part of determining the nutritional and health status of an athlete. The focus of a dietary assessment is to attain the most accurate report on the type, amount, cooking method, and time of consumption for all food and beverages. The ability to properly assess the nutrient intake of an individual entails precise reporting. Choosing the correct assessment method for the athlete being examined ultimately promotes accuracy when analyzing the record.⁴

However, there are many factors to consider when beginning an assessment. One must consider the time available to perform the assessment, the amount of money available, and the reason for the assessment, such as trying to determine specific nutrient intakes or looking for a sign of deficiency. Some of the most important issues that the dietitian needs to be aware of and that will affect an accurate assessment include under- and overreporting food intake, the ability of an athlete/client to be open in reporting, descriptive explanations of food intake, reporting all snacks and beverages, and determining the proper assessment tool and the amount of time to be able to accurately assess nutrient status.

TABLE 1.1
Comparison of Dietary Assessment Methods

Method	Description	Advantages	Disadvantages	Applications
Diet Records	Individual writes down all beverages and food consumed each day for a specified period of time. Portion sizes are either measured or estimated.	Acceptable accuracy and increased compliance. Follow-up interview further increases accuracy.	As time period lengthens, participant compliance decreases.	Usually recorded for 1–7 days, including both weekdays and weekend days.
24-Hour Dietary Recall	Individual describes in detail all the food and beverages consumed in the past 24 hours.	Easy to administer and little burden on individual. Fast to complete.	Dependent upon individual’s memory. May not represent usual food intake. Requires an experienced interviewer.	Used to rank food or nutrient groups. Can be performed on groups of people.
Food Frequency Questionnaires	Given a predetermined list, an individual states the frequency of consumption for the foods on the list.	Can be self-administered. Inexpensive and may represent usual dietary intake. Can provide quantitative information.	Can be population specific. Reliant on the individual’s memory and ability to quantify food intake over a specified period of time. Each questionnaire requires validation.	Used to measure or rank specific nutrients of food intake. Can be used with other methods to as a cross-check.

Source: Adapted from Magkos, F. and Yannakoulia, M., Methodology of dietary assessment in athletes: Concepts and pitfalls, *Curr. Opin. Clin. Nutr. Metab. Care* 6(5), 539–49, 2003.

1.2.4.1 Misreporting

Misreporting is a problematic issue associated with assessing dietary intake and can present itself as overreporting or underreporting in food recalls, diaries, and questionnaires.⁵ Overreporting occurs when an individual claims to consume more foods, whether they are nutritious or nonnutritious, than he or she actually consumes. Individuals who overreport are commonly those who may not consume

fruits and vegetables as recommended,⁶ those who are of low-socioeconomic status, and those with eating disorders. Of the nutritional components, protein is one of the most overreported.⁷

Overreporting, however, is not as problematic as underreporting,⁵ which occurs when an individual does not record or report all of the food that was consumed for that recording period. Individuals and athletes who are overweight are the most prone to underreporting. However, it is important to note that when assessing a food recall, diary, or questionnaire, the most prevalent underreported components are total energy, carbohydrates, and high-fat foods.⁷

Understanding the concepts of misreporting can provide a better understanding and awareness of susceptible populations and ultimately provide a more accurate diet assessment. It should also be recognized that women are 10% more prone to misreporting than men.⁸ More specific to athletes, those involved in weight- and body-focused sports are more prone to misreport energy intake.⁹ Inaccuracy in reporting food intake can cause many problems and lead to false analysis of dietary intake. Because of this, it is important to determine how to detect and counteract these issues. The first step relies on the dietitian's ability to interview and record recalls. Previous experience and the willingness to ask detailed questions and lead the conversation in a way that will elicit proper recollection and reporting of food intake will improve accuracy. The dietitian should also acquire the athlete's usual intake instead of just a day's intake. The usual intake is a combination of several days of daily intake and consists of both weekdays and weekends. A second step that can be taken toward counteracting inaccuracy in dietary analysis includes tests involving determination of urinary biomarkers and the doubly-labeled water technique. These test methods are used as a means to determine nutrient status of certain nutrients. Urinary biomarkers tests can determine protein, sodium, and potassium status. The 24-hour urinary excretion of these nutrients is also able to reflect the difference in energy intake resulting from various levels of physical activity, and this is an important determinant of energy expenditure and therefore energy intake.¹⁰ Doubly-labeled water is also a means to measure energy expenditure and can be used in combination with dietary intake data to determine the energy intake compared to expenditure and determine if over- or underreporting is a problem.¹¹ However, these methods can be costly and difficult to perform with a large group. The best use of these assessments may be of use with "special issue" clients and athletes who need specific health and dietary attention.

1.2.4.2 Snacking

Snacking is an important aspect in understanding the complete dietary assessment. The importance of snack food contribution to the overall energy and nutrient intake of the dietary recall, diary, and questionnaire is vital to the accuracy of such records. The National Health and Nutrition Examination Survey (NHANES) (2002) illustrates that underreporting of snacking foods and amount of snacking occurs often. In contrast to the general population, athletes consume about one third of their total daily energy from snacks.¹² The reason for a greater snacking tendency among athletes is an adaption to their high energy expenditure and needs.¹³ It is important for athletes who expend great amounts of energy to include snacking in their daily

routine but be sure to report it in dietary assessment. Be sure athletes are choosing snacks that are nutritious and that they time intake in accordance with training and competition schedules. This will lead to improved health and performance. Snacks should never be used as a meal replacement but rather serve as an addition of food to the diet. For athletes, the act of snacking may take precedence over other meals, so it is important to make sure reporting is truthful.^{14,15}

1.2.4.3 Openness in Reporting

To ensure that an accurate nutrient assessment is obtained, one of the most essential factors to consider is the relationship between the client/athlete and the dietitian. The more comfortable the athlete is, the greater the response and the more willing he or she will be in reporting the foods and the amount of foods consumed. The dietitian must keep in mind the culture, socioeconomic status, religion, and eating behaviors of the athlete during the assessment. Dietitians must also be aware of any subconscious negative feedback, whether it is a facial expression, comment, or action that would make the client/athlete uncomfortable and unwilling to respond accurately to future assessments. With proper technique and instructions, enhanced accuracy will be ensured.

1.2.4.4 Time Frame for Determination of Nutrient Status

The ability of a food record and 24-hour food recall to precisely and reliably assess macronutrient and energy status takes more than one recall. The U.S. Committee on Food Consumption Patterns recommends that at least four 24-hour recalls be collected during the course of a one-year period.^{4,16} Diet records collected for a 3- to 4-day period are considered an appropriate time frame. However, accuracy is increased with each additional day but only up to seven extra days. This is because the longer time that the client/athlete is required to record, the more likely misreporting and nonreporting become.¹⁷

When attempting to determine a few major contributing nutrients, the number of days of analysis varies for each vitamin and mineral. Other considerations include bioavailability, vitamin and mineral supplements, and foods consumed in conjunction that may affect absorption of one another. The most reliable estimates are obtained within 10% of the obtained usual intake. Estimates within 20% are also used, but not as accurate. Determining an individual's nutrient status can be done by looking at the nutrient intake and comparing it to the Dietary Reference Intakes (DRIs). DRIs, according to the Institute of Medicine, are nutrient reference values based scientifically on provision of good nutrition.

It is important to realize that obtaining accurate nutrient assessment through dietary assessment may not be plausible for certain nutrients; for example, there are better ways in which the status of vitamins A and C can be determined. Therefore, it is necessary for one to be patient when involved in the nutrient assessment process and to report consistently.

1.2.4.5 Dietary Assessment vs. Clinical Testing

An alternative to assessing nutrient status of a client/athlete is the determination through blood and urinary biomarkers. Even better yet is their combination. A

TABLE 1.2**Average Number of Days for Dietary Intake Assessment to Accurately Determine Nutrient Status in Men and Women**

Nutrient	Men (Number of Days)	Women (Number of Days)
Total Energy	27	35
Carbohydrate	37	41
Fat	57	71
Protein	36	48
Iron	68	66
Calcium	74	88
Vitamin A	390	474
Vitamin C	249	222
Potassium	34	48
Sodium	58	73

Source: Adapted from Basiotis, P.P., Welsh, S.O., Cronin, F.J., Kelsay, J.L., and Mertz, W., Number of days of food intake records required to estimate individual and group nutrient intakes with defined confidence, *J. Nutr.* 117, 1638–41, 1987.

combined food frequency questionnaire and urinary nitrogen test may be more reliable than a 24-hour recall and a blood biomarker test in determining nutrients, yet both blood and urinary biomarker tests may be more accurate in assessing nutrients such as β -carotene and folic acid but not protein or α -tocopherol.¹⁹ As noticed here, different nutrients may be better assessed in various ways. If an assessment is being made for a client/athlete who may need close attention, it would be beneficial and accurate to perform both recall and biomarker tests. Similarly, because of the possible misreporting issues concerning dietary recalls, diaries, and questionnaires, collection of blood, urinary biomarkers, or both, serves as a means to validate or to require further assessment of an individual.²⁰

1.2.5 EXCHANGE LISTS SYSTEM

The exchange lists system was developed in 1950 by the American Dietetic Association in conjunction with the American Diabetic Association and the U.S. Public Health Service. This educational tool was originally created for individuals with diabetes to assist them with carbohydrate counting and meal planning, so that they could enjoy consuming a wide variety of foods while balancing their glucose and insulin levels. The system groups foods into three main categories (some of which are further divided into subgroups) according to their nutrient and energy content: carbohydrates, meat and meat substitutes, and fats. Any food item from a particular list can be exchanged for another (with the corresponding equivalent portion size) in the same list because they have similar amounts of calories, carbohydrates, proteins, and fats.^{21,22}

Even though this tool was designed for diabetic patients, it could also be useful for the athletic population. Utilization of the food exchanges can provide a fast, approximate assessment of the amount of calories, carbohydrate, protein, and fat present in the diet. Therefore, athletes familiar with the use of the exchange lists could evaluate their own dietary intake, which could possibly translate into more accurate reporting to their sports dietitians. The use of a Microsoft Excel™ spreadsheet could further simplify the calculation of the amount and distribution of different exchanges throughout the day and their contribution to macronutrient and caloric intake, as well as the determination of each macronutrient's contribution to the total amount of calories consumed, expressed as percentages. These calculations could be very useful in diabetic athletes as well as their sports dietitians, who may choose to use this simple method as a first step in the energy and macronutrient assessment of these individuals. Moreover, the exchange lists may be of special advantage for those athletes traveling abroad, not only to estimate their own dietary intake but also to plan meals according to their sports dietitian's recommendations.

1.2.6 DIETARY ASSESSMENT ABROAD

It is important to take into account that dietary assessment of individuals can vary not only due to the method of assessment employed and its accuracy but also due to ethnic differences. Consider, for example, individuals, especially international-level athletes, who travel abroad for competition. Other countries may have a different food supply, availability, forms of food preparation, serving sizes, and serving format, among others; therefore, sports dietitians analyzing athletes' diets should pay attention to these discrepancies in order to obtain the most accurate dietary assessments possible.

Moreover, specific considerations need to be taken into account when assessing the dietary intake of collegiate athletes who come to the United States from other parts of the world, carrying with them their traditional practices regarding food consumption. For instance, when conducting the dietary assessment of immigrant groups in Europe, researchers were faced with several challenges, including the quantification of specific portion sizes of traditional foods and dishes (such as eating from a shared serving dish/pot vs. an individual plate), scarce information on ethnic dishes and recipes, and composition of culture-specific foods, among others.²³ In order to account for these differences, the method used for dietary assessment of individuals of different ethnic origins should reflect their culture and tradition. However, literature on dietary habits and dietary assessment methods appropriate for different ethnic groups in Western society is limited.²⁴

Consequently, there are several things that international networks, such as the European Micronutrient Recommendations Aligned and the International Confederation of Dietetic Associations, could do in order to facilitate a more accurate assessment of ethnic groups or individuals traveling to countries with different cultural traditions regarding food consumption:

- The standardization of food composition tables or software (to translate food consumption into specific nutrient intake) and homogenization of assessment methods in Europe²⁵ could ease the comparison between European

countries, and extension of this homogenization across the globe would further enhance the ability to account for the above-mentioned differences between people of different ethnicity.

- The development of new food-composition tables and computer databases appropriate for use in various countries and regions (since they are lacking, are outdated, or are incomplete in many countries) would allow for the correct interpretation of data collected from dietary studies around the world.²⁶
- A picture book with country-specific dishes and common restaurant foods typical of different nationalities could be used for the quantification of portion sizes, in order to obtain more accurate assessment of nutrients from the diet.²⁷
- Repeated 24-hour recalls and questionnaires on meal patterns and purchases of foods could be used to assess information regarding food composition and distribution patterns of meals prepared at home, food composition and selection of foods prepared at restaurants, household portion sizes, and food patterns at festival days and on special occasions, as well as seasonal influences, in order to provide a more accurate diet assessment of different ethnic groups.²⁴
- The creation of culturally sensitive dietary questionnaires for athletes of different ethnic origins may also contribute to the specific factors previously mentioned in order to obtain a better nutrient analysis from the diet assessment of such special populations.²⁸

The creation and implementation of the above-mentioned suggestions would be beneficial for sports dietitians in the dietary assessment and analysis of athletes who travel abroad for competition and for international athletes living in the United States. The availability of culturally sensitive dietary questionnaires, including images of common foods from different nationalities, would allow sports dietitians to conduct a more specific and accurate dietary assessment of ethnically diverse athletes and those competing abroad. In addition, the availability of complete food-composition tables, nutrient analysis databases, or software that includes updated information from household and typical restaurant foods from other countries would allow sports dietitians to obtain a more specific and accurate report when interpreting the nutritional status of athletes of different ethnicities.

It is also interesting to note that different U.S. nutrient standards are used around the world to aid in the determination of nutrient adequacy. For example, a Brazilian study on the assessment of water and nutrient intake of adolescent athletes used the DRI and the American College of Sports Medicine (ACSM) guidelines to evaluate the nutritional intake of adolescent athletes.²⁹ Furthermore, the Recommended Dietary Allowances (RDA) were used for the majority of European surveys to compare micronutrient intake of Europeans²⁵ and to estimate nutrient adequacy for individuals and populations.³⁰

These guidelines serve as a reference for European countries to compare nutrient adequacy from the dietary intake of individuals and populations, although they should be adapted to the specific characteristics of European nutrient intake. However, Europe has not yet adopted standardized tables due to variability in the

evaluation of nutritional status among European countries and the lack of knowledge regarding the variability of specific nutrient intake in the population, as well the lack of consensus regarding the type of food consumption database that should be employed to obtain this kind of information.

In addition, there are similarities in the methods of assessment utilized in the United States compared with other countries. Dietary surveys conducted across Europe, as well as studies performed in South America, Africa, and Asia, used food records, food frequency questionnaires, 24-hour recall (single or repeated), dietary history, and combinations of these dietary assessment methods to register the food intake of their individual populations.^{23,26,28,29,31} These similarities could aid in the comparison of individual dietary intakes across the world. Commonalities and differences among these individuals from diverse countries could be used to establish worldwide nutrient databases.

1.3 SPECIAL ISSUES WITH ASSESSING FOOD INTAKE IN ATHLETES

For a more complete and comprehensive assessment of an athlete's diet, special consideration must be given to issues related to periodization (cycles of training or eating), fluid intake, vegetarian diets, gastrointestinal (GI) issues, supplements, traveling, and weight management. Athletes are often unaware of the importance of these matters and may not consider these when reporting their diets. The sports dietitian will need to take additional time with athletes to determine if any of these issues have an effect on the athlete's dietary intake.

1.3.1 PERIODIZATION OF TRAINING AND DIETARY PERIODIZATION

Generally speaking people do not consume the same foods and drinks day after day. Even though a pattern may be followed throughout the week, people may change it during the weekends. This is why dietitians instruct clients to record at least one weekend day when gathering multiple-day dietary records. Moreover, food cost, availability, ethnic background, and family traditions, among other factors, can influence the types and amounts of food and beverages people consume throughout the year, which increases the difficulty of conducting accurate dietary assessments.

Besides these factors, sport dietitians need to take into account periodization patterns specific to each sport when assessing dietary intake of athletes. They follow specific training patterns during the "in" and "off" seasons, as well as during the pre- and postcompetition periods, which are accompanied by different nutritional needs that should be considered during their dietary assessment.

In this context it is important to discuss the athlete's engagement in periodization of training. This method of training was first applied in the 1940s due to the discovery by Soviet sport scientists that sports performance could be enhanced by varying the training loads during the year instead of by maintaining a constant training stress. The implementation of this model of training has been supported by several research studies^{32–34} as well as by the ACSM.³⁵

Periodization involves different training cycles, including load cycles, recovery cycles, peak cycles, and conditioning cycles, that are implemented according to the

athletes' sports demands and schedules of competition.³⁶ The load cycles are considered the building portion of the program and take place during the off-season, in the precompetitive period. The recovery cycles focus on providing the athlete with active rest periods that serve as a transition between the building and competitive phases. Each recovery cycle helps the athlete to be prepared for the following, more demanding peak cycles. The peak cycles are designed to promote maximal strength gain, while allowing time to work on motor skills specific to the sport. The peak cycles are implemented during, or immediately prior to, the competitive period and last approximately the same amount of time as the load cycles. After the competitive season, athletes engage in conditioning cycles, which are periods of active rest that allow athletes to rest from heavy training while avoiding deconditioning.

The implementation of these cycles allows coaches to separate the annual training plan into three main periods: preparation (load cycles followed by recovery cycles and peak cycles), competition (peak cycles), and transition (conditioning cycles and recovery cycles). Following this pattern an athlete will be trained to achieve peak performance during the competitive season. Since each of the cycles differs in intensity and type of training, the nutritional demands for each period would also be different. In order to account for the specific nutritional status of athletes throughout the year, dietary assessments should be conducted at each of the different training/competition periods.

Another consideration to be noted with athletes is that different sports require athletes to compete all year long, while others only require them to compete intensely during one season. Consider, for example, college or professional tennis, which is played on an all-year-round basis, compared to college or professional football, which is only played during the fall season. The energy and nutritional requirements of these two groups of athletes would be different during training, precompetition, and postcompetition periods and would require sports dietitians to account for these disparities when conducting diet assessments. Even comparing football, which is played during the fall, and baseball, which is played during the spring and summer, will require athletes to have different nutritional plans that are most suitable to the sport and time of the year in which they are played. Also, individual sports, such as tennis, compared to team sports, such as football, will place different demands on the athlete's energy expenditure and nutritional requirements, which should be recorded by sports dietitians during the diet assessment process. It is very important that the sports dietitian, as well as every other member on the athlete's health professional team, be familiar with the physical demands and schedules of different sports, such as those listed on [Table 1.3](#).³⁷

In addition to periodization of physical training, dietary periodization needs to be taken into account when conducting dietary assessments. Dietary periodization refers to the manipulation of energy-yielding nutrients obtained from the diet that are related to the changes the athlete undergoes during the different periods of training. These dietary manipulations have an impact on fuel utilization during exercise, specific to the period of training and the sport the athlete performs.³⁸ One such manipulation used by sports dietitians to increase athlete's muscle fuel storage and subsequent supply, especially during events lasting over 90 minutes, is carbohydrate loading. Take, for example, a distance runner, who during the peak cycle (right

TABLE 1.3
Periodization of Diverse Sports

Sport^a	Preseason Training^b	Competitive Season^c	Active Rest^d	Postseason Training^b
Baseball	November–January	February–June	3–4 weeks	August–October
Basketball	August–October	November–March	3–4 weeks	May–July
Cross Country	August	September–November	Most compete in indoor/outdoor track seasons	July–August
Football	June–August	September–December	4–6 weeks	February–May
Golf	July–August	September–May	2–3 weeks	June
Gymnastics	October–December	January–April	3–4 weeks	June–September
Ice Hockey	August–September	October–March	4–6 weeks	May–July
Indoor Track and Field	September–November	December–February	Most compete in outdoor track seasons	Most compete in outdoor track seasons
Outdoor Track and Field	N/A (most have already competed during indoor track season)	March–June	3–4 weeks	July–August
Soccer	June–July	August–December	4–6 weeks	February–May
Softball	November–January	February–May	3–4 weeks	June–October
Swimming	July–September	October–March	3–4 weeks	April–June
Tennis	July–August	September–November January–May	2–3 weeks	June–July
Men's Volleyball	August–September	October–May	3–4 weeks	June–August
Women's Volleyball	June–August	September–December	3–4 weeks	February–May
Wrestling	September–October	November–March	4–6 weeks	June–August

^a All sports include men and women unless specified.

^b The differences in pre- and postseason activities are associated with the training cycle and the type of conditioning that will be performed. The postseason of most athletes focuses on developing strength, power, flexibility, and agility. The preseason focuses on developing more technical skills and specific movement and conditioning.

^c Competitive seasons vary by age group and skill level. The seasons listed here reflect the calendars of most high school and collegiate sports in most states, governing bodies, and associations.

^d Active rest periods vary by age group, skill level, and whether the athlete also competes in other sports. Active rest allows the athlete to recover while engaging in other activities.

Source: Ballew, C. and Killingsworth, R.E., *Nutritional Assessment of Athletes*, 1st ed., CRC Press, Boca Raton, FL, 2002, p. 27.

before competition) may be advised by a sports dietitian to engage in carbohydrate loading in order to increase muscle glycogen storage and improve performance during the race. This dietary periodization would result in an increase of the athlete's carbohydrate consumption compared to the off-season or other periods of training, where high carbohydrate consumption may not be emphasized.

Both training and dietary periodicities used by athletes should be taken into account during the dietary assessment process. Since these manipulations are ongoing, sports dietitians should attempt to gather dietary intake data (using any individual or combination of the methods discussed in the previous section) from the different exercise and dietary periodization phases. Consequently, specific eating patterns could be identified for each of the periodization phases to determine whether the athlete is able to follow the diet recommendations and to assess whether these recommendations are effective in keeping the athlete healthy, as well as in helping him or her meet the energy and nutrient demands of training and competition. Being unaware of the athlete's schedule regarding periodization could result in a misinterpretation of the dietary assessment. Following the distance runner example, not knowing that this athlete is in the peak cycle of training, following a carbohydrate-loading diet in preparation for an upcoming race could result in the misinterpretation that the athlete is consuming inadequate amounts of fats and proteins.

In order to ensure proper dietary assessment planning and gathering of dietary intake data at each periodization cycle, sports dietitians need to be familiar with the timing of the training periodization and calendars of the diverse sports (Table 1.3) as well as with any specific dietary periodization associated with the different training cycles or competition periods.

1.3.2 FLUID INTAKE

Sports are very diverse in nature, and the individuality of athletes performing these athletic events in addition to the variability in training practices results in a great variability in the fluid needs of the athletic population. Fluid intake needs can also vary between practice and competition, and they can be affected by different environmental conditions as well as by the degree of acclimatization of the athlete.³⁹ Therefore, sports dietitians should be aware of the specific needs of the athletes they are working with and take into account these variables to ensure proper fluid recommendations. The assessment of an athlete's fluid intake helps monitor whether the recommendations are adequate. This is especially important since the majority of athletes do not drink enough to replace their loss of body fluids through sweat during exercise,⁴⁰ which can have serious consequences in health and athletic performance. What is more, several athletes appear to avoid drinking, although they recognize that proper rehydration is likely to enhance their performance.⁴⁰

However, fluid assessment through dietary recalls or food records may not be very accurate, since athletes commonly underreport fluid intake.¹ One of the reasons for fluid underreporting may be that it is difficult to quantify the amount and composition of fluids consumed during training/practice sessions as well as during competition, since athletes may consume copious amounts of sports drinks with added carbohydrate and electrolytes, which would have different implications in the

total dietary assessment compared to water. Also, unless athletes are thoroughly interviewed by a sports dietitian during a 24-dietary recall, for example, they may not remember when and how much fluid they have consumed, resulting in under-reporting of fluid intake from diet records. In addition, another reason for under-reporting of fluids consumed throughout the day may be that athletes as well as nonathletes rely on the thirst mechanism and consequently may not be consciously thinking about drinking water and other fluids. Moreover, fluids that are consumed in between meals are more likely forgotten to be reported in diet records than fluids consumed with meals.^{7,41}

The assessment of hydration status is a critical component to ensure complete rehydration of athletes engaged in regular and intense training and competition in hot environments. However, there is controversy in fluid balance science regarding the selection of a proper hydration assessment method.⁴² There is a lot of variation in the applicability of the different hydration assessment methods due to methodological limitations; for instance, the presence of needed measurement conditions (reliability), the simplicity and cost of implementation (simplicity), the sensitivity for discovering small but significant changes in hydration status (accuracy), and the type of dehydration anticipated^{42,43} are all valid aspects of study. In this context, the estimation of an individual's or a population's fluid needs can be obtained through dietary recalls, records, or surveys providing qualitative data; however, data quantification is difficult using dietary assessment. On the other hand, water balance examination and biochemical assessments provide quantitative data to aid in the correctness of reported intakes. The combination of plasma osmolality and total body water (measured by isotope dilution) is the "gold standard" for assessment of hydration status.⁴³

Other hydration assessment methods include total body water estimated by bioelectrical impedance analysis; plasma markers other than osmolality, such as sodium, changes in hematocrit and hemoglobin concentrations, or changes in the concentrations of hormones involved in body fluids regulation; urine markers, such as specific gravity, osmolality, or color; and body mass changes. In addition, other variables such as salivary measures and physical signs and symptoms of clinical dehydration can be considered.

The gold-standard methods for assessment of hydration mentioned above are the most precise, involve substantial methodological control, are expensive, and require analytical expertise in order to utilize them. Therefore, they are more suitable for application in sports science or medicine, or for determining reference criteria and use in research, but they are not practical to employ in the daily monitoring of hydration status of athletes during training or competition. More practical methods to use in this situation that are sufficiently sensitive to identify daily divergences from euhydration (the normal state of body water content) include measurements of body mass changes combined with a measure of urine concentration taken from the first urination of the morning. These techniques are easy to use, are inexpensive compared to the gold-standard techniques, and can provide accurate distinction between euhydration and dehydration—thus they can be utilized as an individual source for assessment.^{44,45} On the other hand, methods such as plasma markers (except for osmolality), bioelectrical impedance analysis, saliva measures, and clinical physical

signs and symptoms of dehydration are frequently confounded or not sufficiently accurate to assess an athlete's hydration status with reliability.^{46–49}

As mentioned above, simple markers for hydration assessment such as body mass changes and urine concentration measures are suitable for use with the athletic population. The body mass technique is usually used in the laboratory and field environment to determine rapid changes of hydration in athletes. These changes are calculated as the body mass difference between pre- and postexercise. It is best to state the level of dehydration as a percentage of the pre-exercise body mass instead of as a percentage of total body weight, since the latter varies significantly.⁴³ Interpretation of the results from this method indicates that 1 g of lost mass is equivalent to 1 mL of lost water. This method focuses in the determination of water loss as a measure of hydration status, but it fails to explain metabolic carbon exchange, which denotes the lone small error in this assumption.⁵⁰

Furthermore, in the laboratory setting, this body mass method is frequently used as a standard against which the resolution of other hydration assessment markers is compared. Indeed, there is evidence that body mass changes that are accurately controlled can offer a more sensitive estimation of acute changes in total body water than repeated measurements by dilution methods.⁵¹ The use of this method in endurance athletes during a race, for example, is particularly helpful in deciding whether the athlete's symptoms are due to dehydration or overhydration (the latter is usually associated with symptomatic hyponatremia⁵²), which would impair health and performance. Moreover, changes in body mass may be a satisfactorily stable physiological marker to examine daily fluid balance over long periods of time (1–2 weeks), helping athletes with acute fluid changes and undergoing hard exercise to maintain a stable body mass by compensating for sweat losses estimated by this method.^{53,54}

However, over longer periods, chronic energy imbalance can result in changes in body fat and lean mass tissues, which would be manifested as changes in body mass and would consequently be a limitation to this hydration assessment method. In such case, body mass measurements should be employed in combination with another hydration assessment method, such as a urine concentration test, to distinguish between tissue and water losses. Urinary markers indicative of dehydration include reduced urine volume, high urine specific gravity, high urine osmolality, and dark urine color. These markers are simple to measure and they provide a reliable assessment technique to distinguish between euhydration and dehydration,^{44,45} as long as the urine sample used for assessment is obtained from the first urination in the morning following an overnight fast.⁴⁵

Urine samples taken under other conditions may not be as accurate. In fact, they have a poor correlation with plasma osmolality (one of the gold-standard methods for hydration assessment), failing to consistently monitor documented changes in body mass corresponding to acute dehydration and rehydration.⁵⁵ It seems that there is a delay in the promotion of endocrine regulation of the reabsorption of renal water and electrolytes by plasma osmolality changes at the kidney when acute changes in body water take place.⁵⁵ It is also probable that composition of the consumed drinks has an effect on this response. It has been shown that abundant urine production appears much earlier than the time at which euhydration is reached when drinking large volumes of hypotonic fluids.⁵⁶ The measurements of urine concentration may also

be altered by diet.⁵⁷ Nevertheless, analysis of a urine sample obtained from the first urination in the morning after an overnight fast reduces confounding influences and increases the reliability of this method.⁴⁵

For practical purposes, sports dietitians should encourage athletes and their coaches to monitor daily fluid balance using the latter two simple methods (first-morning urine samples and body mass measurements). This can be accomplished at a relatively low cost and with easy-to-use commercial instruments, such as urine specific gravity and conductivity (osmolality equivalent) assessment tools, as well as urine color charts.^{44,45,58} Regarding body mass change measures, a kilogram scale or a medical-grade scale manufactured according to international weighing standards would be the preferred choice. However, almost any scale can be suitable for athletes to self-monitor their body mass changes as long as they measure their nude body mass. Again, these two methods, body mass changes and urine concentration tests, are simple hydration assessment methods that provide sensitivity for identifying important differences in fluid balance (above 2% of body mass change) for athletes during training and competition.

There is still a simpler approach recommended for self-monitoring of daily hydration status in athletes. This approach is represented in Figure 1.1. It uses the pneu-monic WUT, which combines three of the simplest markers of hydration: weight, urine, and thirst (WUT).⁵⁹ By itself none of the three markers provides sufficient evidence of dehydration, but the combination of any two indicates that dehydration is likely, and dehydration is very likely when all three markers are present. This method was established in accordance with reliable scientific principles of hydration assess-ment.⁵⁹ However, since the intention of this concept is to provide an easy way to assess hydration, it only requires a body-weight scale. In case dehydration is suspected and using WUT and following fluid intake recommendations does not result in restoration of euhydration, then other, more precise assessments should be conducted.

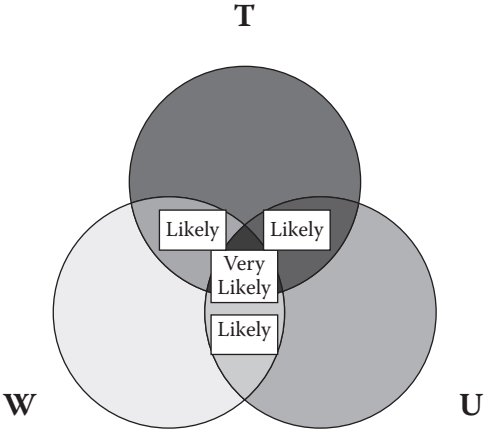


FIGURE 1.1 Markers of hydration: Weight, urine, and thirst (WUT) athletic hydration assessment tool. (Adapted from Cheuvront, S.N. and Sawka, M.N., Hydration assessment of athletes: “WUT” is the answer?, *Sports Science Exchange* 97 Suppl., 18(2), 11–12, 2005.)

One of the three assessment components of WUT is weight, which should be measured as nude weight, first thing in the morning. Body weight losses in excess of 1% measured on a day-to-day basis may suggest dehydration. This is a day-to-day loss of 1.54 lbs. (0.7 kg) for an athlete weighing 154 lbs. (70 kg). Athletes should combine the information from this measurement with the changes in urine or thirst measures in order to achieve a more certain parameter of dehydration. The second assessment component of WUT is urine. Indications of dehydration from urine are a diminished daily urine frequency and darkening of urine color from a sample obtained at the first void of the morning. Again, athletes should combine the information from this measurement with information about thirst and changes in body weight in order to be more certain regarding the possibility of being dehydrated. The last component of WUT is thirst. The existence of thirst indicates dehydration and the necessity to drink. Thus, if athletes experience thirst, they should combine this information with that from the body weight and urine measures to determine with more certainty if they are indeed dehydrated.⁵⁹

Sports dietitians should encourage athletes and their coaches to use these hydration measurement tools in order to monitor and maintain a proper fluid balance that will sustain health and aid in performance. Sports dietitians can explain these assessment techniques to athletes and coaches, giving them specific tips to help them assess their hydration status from the discussed measures. For example, they can promote the use of charts where athletes can record their body weight, thirst, and urine color, stressing that the loss of 1% of body weight, the presence of persistent thirst, and dark-colored urine are possible indicators of dehydration.⁵⁹

Sports dietitians may also want to educate athletes about how to record and analyze these measures. For instance, in order to examine how much fluid athletes lost or gained during a practice session or in a competition event, they need to record their nude body weight to the nearest pound before and after the exercise. If they lost an excess of 1% of their body weight, they did not consume enough fluids during exercise; on the other hand, if they gained weight, they consumed an excess amount of fluids. In order to help athletes estimate how much fluid they should consume to reestablish euhydration, sports dietitians could instruct athletes to add the amount of weight they lost during exercise (in ounces) to the amount of fluids they consumed during exercise (in fluid ounces); the value obtained from this addition would be approximately equal to the amount of fluids athletes should consume after exercise to replace their sweat losses.

1.3.3 VEGETARIAN DIETS AND ASSESSMENT

Vegetarian diets can be healthful and are able to provide adequate nutrition in regard to the athletic population. A vegetarian diet is one that excludes meat, including fowl and seafood, and products containing those foods. A lacto-ovo-vegetarian excludes all meat products but consumes eggs and dairy products. A lacto-vegetarian excludes meat products and eggs from their diet. A vegan is similar to a lacto-vegetarian; however, vegans exclude all animal products, including meats, eggs, and dairy.⁶⁰ Athletes, especially during high physical activity, need to meet their macronutrient needs, particularly carbohydrates and protein, to replenish glycogen stores, maintain body

weight, and repair as well as rebuild muscle tissue.⁶¹ Protein consumption through the diet is recommended and encouraged prior to taking additional supplements. Planned vegetarian diets can meet an athlete's current protein recommendations through the diet alone without the use of protein supplements. Although the plant proteins are not as bioavailable, it is possible for the vegetarian athlete to meet his or her protein needs through well-planned meals. Vegetarian athletes may need to increase their protein needs to approximately 1.3–1.8 grams per kilogram of body weight.⁶⁰

Iron status needs to be addressed when working with vegetarian athletes. Low iron status can have an effect on performance, muscle function, and work capacity.⁶¹ After a thorough nutritional assessment, a dietitian may suggest a blood test to determine iron status. Vegetarian athletes may need to consume an elevated amount of iron compared to their carnivorous counterparts to meet their RDA. An iron supplement may be beneficial after continued contact with the athlete and monitoring of his or her iron status. Other nutrients that are found in meat products, such as vitamin B₁₂, riboflavin, vitamin D, calcium, and zinc, need to be met through other sources. A sports dietitian can assist vegetarian athletes with high-quality plant protein combinations as well as other sources, such as eggs and dairy, where these nutrients can be found.⁶⁰

A concern with vegetarian diets, specifically seen in women athletes, is the avoidance of meat to assist in decreasing caloric intake. If an athlete changes his or her diet and becomes vegetarian, it is important to monitor the athlete's energy intake and body weight as well as body composition. A move toward vegetarian diets has been a sign of disordered eating, which is one aspect of the female athlete triad.

1.3.4 GASTROINTESTINAL ISSUES AND ASSESSMENT

Celiac sprue disease is a genetic condition when an athlete cannot digest gluten, thus causing GI issues, and this can lead to other complications such as weight loss. The small intestine becomes damaged due to the immune response because the gluten cannot be broken down appropriately. Symptoms of celiac disease include abdominal pain and bloating, chronic diarrhea, vomiting, constipation, and pale, foul-smelling stools.⁶² Without proper care the small intestine eventually will not be able to absorb nutrients, thus leading to malnutrition. Celiac disease is a highly individualized disease; therefore two athletes will have different tolerances to gluten. Gluten, a protein that is found in oats, wheat, rye, and barley, can be found in many foods and objects; athletes will need to be aware of what they are consuming and the products they are using. Due to its overwhelming presence in foods, an athlete with celiac disease will need nutritional counseling to assist in food choices as well as any changes that can be made in his or her diet.

Crohn's disease is a condition of the digestive tract. This disease can be seen in any area of the GI tract but is usually found in the small intestine, specifically the ileum. The area that is affected by the disease is inflamed and can cause pain as well as frequent expulsion of waste, leading to diarrhea. This disease has very similar characteristics as other bowel diseases, and an athlete will need testing to determine a specific diagnosis. Malnutrition can also form due to this disease. It is important for the athlete to meet with a dietitian to determine possible deficiencies and dietary

treatment for malnutrition. Again due to the variability of the disease, an athlete with Crohn's disease will need to monitor his or her diet with the assistance of a dietitian to determine the foods that are not well tolerated.

Diverticulosis is a condition where the large intestine forms weak areas that become pouches. These pouches, called diverticula, become inflamed then progress into the disease diverticulitis.⁶³ An athlete can oscillate between diverticulosis and diverticulitis throughout the disease. For the most part, people do not feel much, if any, pain or discomfort with diverticulosis. However, when these pouches become inflamed, symptoms may arise such as changes in bowel habits, lower abdominal pain, cramping, and nausea.⁶⁴ It is suggested that diverticular diseases developed due to the decreased consumption of fiber in the diet. When a stool is caught in the diverticula, this can cause inflammation, thus causing the condition to progress to diverticulitis. After a diagnosis of diverticulosis, an athlete should meet with a dietitian to assist in determining the foods that should be consumed during both stages of the disease.

Irritable bowel syndrome (IBS) is a disorder with symptoms such as abdominal cramping and pain, bloating, constipation, and diarrhea. Even though this syndrome can be painful and possibly debilitating for some, it does not have any detrimental effects in the intestines and does not lead to any serious conditions. Most of the symptoms can be treated through control of an athlete's diet, stress management, and medications. Working with a physician and a dietitian can help an athlete identify possible treatment options as well as different interventions that can be taken to decrease IBS symptoms.

Diarrhea is defined as loose, watery stool. Acute diarrhea can last less than two days and does not require medical treatment. However, chronic diarrhea may cause medical issues and needs to be addressed by a physician. Chronic diarrhea can lead to possible nutritional complications as well as dehydration. When meeting with a dietitian, it is imperative to be as open and truthful as possible about bowel movements, which is covered more thoroughly in the "Openness in Reporting" section of this chapter. Diarrhea is a symptom of many gastrointestinal issues that are listed in this section.

Constipation is a variable condition as well. For some individuals, not having a bowel movement each day would be considered constipation. Constipation is defined as having a bowel movement fewer than three times a week.⁶⁵ Constipation is a symptom, not a disease, and can usually be caused by a poor diet. There are many factors that can lead to constipation, including a lack of fiber in the diet, dehydration, medications, laxative abuse, and changes in a routine.

Lactose intolerance is a common condition where an athlete is not able to tolerate dairy product consumption. Again, this condition is highly variable, and athletes with this condition may be able to tolerate very different dairy food choices. Lactose intolerance is due to the gastrointestinal tract not being able to digest the sugar, lactose, found in dairy products due a lack of the proper enzyme.⁶⁶ Products such as milk, cheese, ice cream, and other dairy products may need to be eliminated from the diet to decrease the symptoms of this condition. Symptoms that are usually seen with lactose intolerance include diarrhea, abdominal cramping, gas, nausea, and bloating. Dairy products such as yogurt may be better tolerated due to the inherent

characteristics of enzymes that can assist in the breakdown of lactose. Meeting with a dietitian can help an athlete determine the food products he or she can consume. Products are currently on the market that include the enzyme lactase, thus athletes with lactose intolerance may be able to consume these products with fewer GI symptoms. Pills and tablets containing the enzyme needed to break down the sugar in dairy products are also available and can be taken prior to consuming dairy products to assist in the digestion of these products.

The above descriptions are only a few of the many different gastrointestinal issues athletes may have or develop. It is important to be aware that everyone has different symptoms and that diseases affect each individual differently. If athletes have any gastrointestinal issues or have concerns about possible genetic issues, it is important to have them visit a physician or a specialist to assist them in gaining knowledge about the specific issue.

1.3.5 SUPPLEMENTS

A dietary supplement, as defined by the National Institutes of Health, is a product taken by mouth that contains a “dietary ingredient” intended to supplement the diet. Such dietary ingredients may include vitamins, minerals, herbs or other botanicals, and amino acids, as well as substances such as enzymes, organ tissues, glands, and metabolites. Supplements are not currently regulated by the Food and Drug Administration (FDA) and are still available over the counter, without a prescription. Unfortunately there are no required tests regarding the safety, efficacy, or purity of dietary supplements before they are put onto the market. The Dietary Supplement Health and Education Act (DSHEA) of 1994 specifically removed FDA and other agency reviews for the sale of these products. However, the FDA reserves the right to remove a product after it has been proven harmful. The DSHEA places dietary supplements under the umbrella of “foods” instead of “drugs” and limits the Food and Drug Administration’s ability to regulate the substances.⁶⁷ For years, there has been considerable hype surrounding the use of many of these supplements.

Supplements may be consumed for health purposes or for their performance-enhancement claims. The total amount of money spent on dietary supplements today is over \$20 billion a year.⁶⁷ Cassileth (2009) reports that about 52% of people consumed one or more dietary supplements on a regular basis in 2009, compared to 46% of people who consumed supplements on a regular basis in 2006.⁶⁷ This is owed in large part to the significant promotion by the media. Not surprisingly, athletes are most likely to use various supplements, especially those that claim to provide a competitive edge.⁶⁸ It is crucial for the dietitian to work closely with the athlete and be aware of possible supplement usage for accurate determination of the dietary assessment as well as for health reasons.

Several physical, emotional, and mental stressors are put upon an athlete. This type of stress on the body can affect health and increase nutritional needs. Especially for those athletes involved in vigorous training programs, consumption of enough food may be difficult, as well as the ability to consume enough nutrient-dense foods. Inability to eat healthfully, in time, can lead to nutrient deficiencies that may affect critical functions of the body and ultimately lead to disease and impaired performance.⁶⁹

Vitamin and mineral supplements can be a beneficial addition to an athlete's daily regimen. Most often, athletes need to be reminded that the best way to get vitamins and minerals is from actual food, and a supplement is just that—a supplement to the diet and not an alternative source. In a well-balanced diet, nutrient needs can usually be met; however, it is important that appropriate probing about the athlete's supplement usage occur since there may also be adverse effects, such as interactions with other nutrients and toxicity from use. When conducting a dietary assessment, any and all vitamin, mineral, and other supplements should be included to account for extra nutrient intake. It is important to be aware of the various vitamins and minerals an athlete is consuming not only for assessment and analysis purposes but also to understand interactions that may occur with other foods or supplements.

Some of the most important vitamins and minerals for athletes include calcium, iron, magnesium, potassium, sodium, B vitamins, and vitamins D, C, and E.⁶⁹ Calcium and iron, in particular, are two minerals for which it is crucial that athletes have adequate amounts. Calcium is necessary for bone health and protection from injury as well as muscle contraction. Iron aids in the transport of oxygen throughout the body and allows oxygen to be taken up into the muscle. Women particularly need to be aware of their iron status, as menstruation reduces iron levels to an even greater degree. Iron deficiency causes fatigue, malaise, difficulty breathing, and dizziness. Iron deficiency for prolonged periods leads to anemia, which causes the heart to have to work harder to pump oxygen through the body.⁷⁰ The other vitamins and minerals noted help keep body fluid levels normal and allow other important metabolic functions to take place.⁶⁹

Protein and amino acid supplements are some of the most popular among athletes. Although they are not a typical food item, protein supplements contain other nutrients and calories that need to be documented on an athlete's dietary assessment. Some athletes may also add fruit and other nutrient supplements to drinks consisting of a powder base with added vitamins, minerals, and various forms of protein, which can include whey, soy, or amino acids. During a dietary recall, be sure to inquire about the use of protein and amino acid supplements and the form in which they are consumed, as well as any other added nutrients.

Use of supplements can be tricky. It is important to evaluate and make sure supplements do not contain any ingredients banned by agencies, conferences, or leagues. Unfortunately, for the case of several supplements, not all ingredients included in a product are listed. Without regulation by the FDA, there is no way to control what manufacturers include in their product; as such, it is often difficult, if not impossible, for the buyer to know. There are numerous reports of professional athletes unknowingly consuming banned substances, ultimately failing drug tests, and in many cases effectively ending their career.⁶⁸

A couple of the most referenced guides to banned supplements in the United States stem from the National Collegiate Athletic Association and the World Anti-Doping Agency. Other countries have very different regulations on supplements and even banned substances. Each country has compiled its own list of banned substances, which can be accessed through its national governing bodies.⁶⁸ Several classes of supplements and medications are prohibited and include stimulants such as caffeine, anabolic agents, alcohol and beta blockers (only for rifle and vehicle racing),

diuretics and other masking agents, narcotics, cannabinoids, peptide hormones and analogues, antiestrogens, glucocorticosteroids, and beta-2 antagonists. It is crucial that the dietitian know about possible supplement use, know which ones may be more popular for some sports over others, and watch for possible signs, symptoms, and side effects of prohibited supplement use.

Herbal agents are quickly becoming another popular supplement among athletes. With the frequency of traveling, athletes are exposed to many different herbal products, especially in China and India.⁶⁸ Use of these products must be under close watch because their contents may include over 30 different herbs per supplement. Oftentimes these herbs may include traces of banned substances or other exotic ingredients that may cause toxicity or adverse side effects. Like their treatment in the United States, herbs and other supplements in foreign countries are not regulated. Ingredients are usually not written in English, and concentrations of each herb may differ from one batch to the next.⁶⁸

1.3.6 TRAVELING

1.3.6.1 Jet Lag

Jet lag is a phenomenon caused by changes in the normal circadian rhythm of the body that affect sleep and wake cycles that occur naturally. Interestingly, there are similar circadian patterns for daily meal times. These food consumption patterns are affected just as the sleep cycle is during jet lag. When there is a change in eating patterns, blood flow to the gut and consequent inadequate absorption may persist. Some of the best ways to counter jet lag are behavioral approaches and timing of food intake. Changing patterns of eating before a long flight, such as the Argonne diet, alter symptoms of jet lag.⁷¹ This diet alters days of feeding and fasting on a protein-rich breakfast and carbohydrate-rich evening meal.⁷² The dietitian must be aware of travel times to understand any changes in feeding patterns and to be able to advise the athlete on staying fueled and hydrated throughout the trip.

An important related concern during travel and prolonged flights is the possibility of dehydration. The air circulating inside the airplane cabin is dry, which causes an increased loss of moisture from respiration. This can be an issue since this may unknowingly affect the athletes' hydration, which in turn may have an impact on performance in outside events in humid and high-temperature climates. Athletes should be advised to consume more water than normal during the travel period, as well as a few days before and after the flight. Intakes of at least 15–20 mL of fluids per hour of flight over and above normal fluid consumption are encouraged.⁷¹

1.3.6.2 Dining Out and Eating on the Road

Traveling can pose some issues dealing with food, nutrient, and fluid consumption of the athlete. Not having access to regularly consumed foods as well as preparation and cooking techniques that the athlete is familiar with may be an issue due to differences in appropriate nutrient intake specifications. Often restaurants use oils, butter, and added sugars to foods to provide flavor. Not all athletes may be used to consuming these extra fats and nutrients in their food, and it may cause gastrointestinal complications and be

problematic for those who are on a restricted diet and watching caloric and specific nutrient intake. Restaurants, take-out, and fast-food venues are the main sources of food consumption for traveling athletes. It can be difficult to control serving size to get the proper amount of food, and more importantly, preparation and cooking techniques are difficult to control, which can alter the healthfulness of a food. Especially during recovery, it is important for athletes to consume carbohydrates and protein to aid in muscle growth and repair. Often, restaurants offer dishes that may not provide adequate amounts of these nutrients or adequate types (high-fat cut of protein versus a lean protein). In these cases, it is important for athletes to bring some snacks and food supplies of their own, such as sports bars, fruits, ready-to-drink protein beverages, nutrient shakes, and any canned foods such as meats, beans, and soups.⁷¹

When dining at a restaurant, choose meats that are grilled or baked and lean sources. For healthy steaks, choose sirloins. When deciding on soups, dressings, and sauces, stay away from creamy ones and choose tomato-, broth-, or oil-based instead. Vegetables are great choices for side dishes, but should be cooked without butter. Oftentimes the restaurant has nutrition facts on their Internet Web page. This information can be used to ensure a more accurate diet assessment. If there is no information available, be specific when questioning and performing the dietary recall. Be sure to ask about preparation techniques and specifics of prepared vegetables and any sauces or dressings used with the meal. It can be difficult for the athlete to know about meals and foods made in other locations, which is where the dietitian must try to extract as much specific information as possible.

When traveling to different countries, athletes must be aware of diverse eating patterns. Different eating times, food preparations, types of foods consumed, and hygiene of food can present various issues for the athlete traveler. Those with a discerning palate may have a hard time adjusting to different tastes. A good consideration for traveling is to investigate the available foods and to also bring along some food supplies from home (making sure they are in line with travel guidelines). Being extremely cautious of exotic foods and preparation techniques is crucial to maintaining health. Exotic foods and use of water may cause adverse effects and subsequent health problems such as gastrointestinal upset and possible viruses. Important tips to remember, depending on the destination, include drinking only fluids from sealed bottles, avoiding ice in drinks, choosing cooked vegetables instead of raw ones and salads, peeling all fruits, avoiding all uncooked foods and unpasteurized dairy products, and not buying food from local markets. Again, in this situation it is necessary for the dietitian to specifically inquire about the foods consumed to obtain an accurate nutrition assessment.^{73,74}

1.3.7 WEIGHT MANAGEMENT

Specific body composition or characteristics are associated with different sports that contribute to the athlete's chances of success in competition. This is due, in part, to certain physiological benefits such as effective thermoregulation or a greater power-to-weight ratio associated with specific body characteristics. Athletes and coaches in many sports, such as wrestling and gymnastics, are usually convinced about the benefits of weight or fat loss, based on personal or anecdotal experience, instinct, or

the false belief in experienced observation of successful athletes. Therefore, athletes striving to achieve these desired body characteristics are often prone to engage in unbalanced, irregular, or very restrictive eating patterns, which can increase their risk of becoming dehydrated or nutrient deficient, or of developing eating disorders.⁷⁵

The presence of these problems and inadequacies in the diet is then translated into the dietary assessment of athletes,⁷⁶ as discussed in [Section 1.2](#) of this chapter. Therefore, professionals assisting athletes with body-type management should be knowledgeable and trained in anthropometry.⁷⁵ In addition, sports dietitians should be familiar with the sport-specific nutrition requirements, body composition standards, and athletes' beliefs toward the standards in order to conduct the dietary assessment process in the most specific and effective way possible, so that interpretation of the assessment's results can help elucidate possible problems in the diet of athletes that could affect both health and performance.

One case of impaired health and subsequent impaired performance in athletes results from recognized eating disorders such as bulimia nervosa and anorexia nervosa. These conditions have specific symptoms that meet the Diagnostic and Statistical Manual on Mental Disorder IV diagnostic criteria. Also, another type of diagnostic criteria that situates athletes at health risk is the Diagnostic and Statistical Manual IV category, which refers to "EDNOS" (Eating disorder not otherwise specified),⁷⁷ meaning that even though some athletes may have a disordered eating pattern, they do not fulfill the characteristics to be considered bulimic or anorexic. The susceptibility and development of eating disorders among athletes may be apparent in athletes attempting to change body type through diet and/or extreme exercise.⁷⁶

This underlines the importance of frequent and well-planned diet assessments in order to detect the presence of eating behaviors to provide early intervention and nutrition counseling to those athletes who may be at risk of developing eating disorders. Dietary recalls and recognized testing modules like the Eating Attitude Test and Eating Disorder Inventory are dietary assessment tools that can definitely contribute to identifying athletes at risk for developing eating disorders that may in turn impair their health and performance.⁷⁶

Another aspect of weight management in need of consideration is that standard measures for body composition used for the general population may not apply to the athletic population. Such is the case of Body Mass Index (BMI), which is especially apparent when comparing BMI values from football players to the general population or even other sports. Desirable body sizes for football players for the different positions in which they play may be considered unhealthy for the nonathletic population. In a study with football collegiate freshmen, the BMI values varied from 23.8 for the quarterback to 33.1 for the offensive lineman.⁷⁸ In addition, the average BMI range for players in the National Football League is from 27.2 for the defensive back to 36.0 for the offensive lineman.⁷⁹ The differences between the BMI values for the various football positions and the body size of football players are partially the reason for which energy needs and body-image perception in collegiate football are different compared to other sports and the nonathletic population.⁷⁶

Many athletes have greater Lean Body Mass (LBM) compared with their nonathletic counterparts. In addition, they are routinely involved in rigorous physical training programs compared to the nonathletes. Therefore, standard calculations for

body size (such as the BMI) and energy needs (such as Harris Benedict or Mifflin-St. Jeor equations) based on parameters for the regular population are not often useful for the athletic population. In the case of some football players who weigh 125% or more of the ideal body weight (based on the standard methods), the resting energy expenditure should be based on their LBM.⁷⁶

There seems to be a tendency in the general population toward underreporting by approximately 20% when assessing dietary intake.^{80,81} Particularly to the athletic population, underreporting of dietary intake has been commonly shown in female endurance runners, gymnasts, divers, and dancers due to dissatisfaction with their body images.^{82,83} This could result in a disparity between energy intake and energy expenditure of athletes, which could in turn have negative health consequences associated with eating disorders, such as bulimia, anorexia nervosa, or EDNOS. However, “decreased energy availability,” which is a chronic inadequate energy intake with or without weight loss, has more recently been recognized as the main cause of amenorrhea and bone mineral loss in female athletes who were otherwise healthy.⁸⁴ On the other hand, overreporting can also occur in the athletic population, since athletes may report not only too low but also too high intakes compared to their real intakes, especially when they know that what they are consuming is unacceptable.⁸⁵ Consequently, experienced sports dietitians familiar with the specific nutritional requirements and anthropometry of the different sports should be able to recognize these discrepancies.

Dietary assessment of athletes is not an easy task. This is due, in part, to the expected inaccuracies in using self-reported data, such as in the case of dietary records kept by athletes. Even considering these inaccuracies in self-reporting, the 7-day food diary seems to be most accurate in the estimation of reported energy and nutrient intake compared to other food records.⁸⁶ Separately, an easy-to-use semi-quantitative food record was compared to total energy expenditure of nonobese subjects estimated by doubly-labeled water technique, and it was found to provide good estimates of energy intake.⁸⁷ However, the comparison of energy obtained from the diet to the recommended intake is limited by the impact of the specific sport, type of training, and individual anthropometrical data, which adds more variability to the interpretation of dietary assessments.⁷⁶

Therefore, in order to have a better estimate of the actual energy consumed by athletes over time, the employment of other methods in addition to dietary assessment can provide more complete and accurate results. For example, biochemical markers such as unexplained low blood glucose or the existence of urinary ketones may imply that the athlete has an energy deficiency.⁸⁸ Observed signs of fatigue, decline in performance, and in the case of female athletes, irregularity in the menstrual cycle, suggest the possibility of energy deficiency that will need to be further investigated.⁸⁴ Anthropometric measures are also helpful, since they can be utilized to monitor changes in lean and fat mass over time. However, due to the error involved in the measurement of anthropometric data, and to the unpleasant feeling that some athletes may experience during this type of assessment, a high level of technical and counseling expertise is necessary for these measures to be safe and useful.⁸⁹ Even though coaches are frequently concerned that tracking body composition in these

athletes will influence them to become more focused on their weight,⁹⁰ failure to do so may result in continuing and undetected energy deficiency.⁷⁵

In addition to these methods, there are yet other methods of dietary assessment available to improve accuracy and reliability. These methods include metabolizable energy intake balance, room calorimetry, indirect calorimetry, heart-rate monitors, accelerometers, and doubly-labeled water. The use of some of these methods may be more pertinent for research purposes due to their high cost and required expertise for utilization. A study of the direct comparison of estimates of daily energy expenditure in healthy adults, using energy intake from 7-day self-reported diet records, metabolizable energy intake balance, and energy expenditure measured by doubly-labeled water and 24-hour room calorimetry showed that self-reported dietary records and room calorimetry underestimated daily energy expenditure. In addition, while energy intake balance provided accurate estimates of energy expenditure, energy expenditure measured by doubly-labeled water was a precise and more direct approach.⁹¹ The results of this study suggested that metabolizable energy intake can accurately estimate energy expenditure, but it has to be done with a controlled feeding and the protocol can be burdensome for the subjects and may even create confounding results due to the interference it causes in the subjects' normal daily activities and eating practices.⁹¹

On the other hand, dietary intake records cause less interference but they have been shown to be inaccurate and to underestimate energy expenditure. The room calorimeter is the most accurate tool to determine energy expenditure during a 24-hour period; however, the results obtained from this measurement are limited to the energy expenditure of a subject restricted in activity to a small chamber. Overall, total energy expenditure measurement using doubly-labeled water is a more direct approach to determining free-living energy expenditure than metabolizable energy intake balance or calorimetry methods.⁹¹

Another study, conducted with endurance runners to assess their total daily energy expenditure with the use of heart-rate monitors resulted in a greater than expected total energy expenditure, which was significantly affected by the athletes' energy expenditure.⁹² The heart-rate method has been widely used in the athletic population,⁹³ and it has been considered to be a more convenient method to be used with athletes when compared to the accelerometers (which were regarded as limited to ambulatory activities and to be inaccurate at running speeds over 9 km/hour) or the doubly-labeled water method (regarded as the most accurate method but inconvenient to monitor energy expenditure of shorter durations).⁹²

Actual energy expenditure obtained from several of the above-discussed methods can be compared with dietary assessment to determine the accuracy in self-reported food intake of athletes. These energy-expenditure methods, as well as anthropometric measures and laboratory analysis data, can also provide an insight about unsafe and inappropriate weight-management techniques some athletes may engage in to meet certain body-type standards. In addition, expertise in the sport-specific nutritional requirements, body-type characteristics, and athletes' beliefs toward them, as well as training in anthropometric techniques, can help sports dietitians determine possible inaccuracies in the dietary intake and dietary-intake reporting of athletes. This in turn can allow sports dietitians to elucidate possible problems in the athlete's

diet or his or her perceptions toward foods and body-type characteristics that could affect both health and performance.

1.4 TRANSLATION OF DIETARY ASSESSMENT INTO ANALYSIS

Registered dietitians have a wide availability of computerized nutritional analysis software, which allows them to obtain individual food composition information as well as to determine caloric and nutrient intake from nutrition assessment reports. The computerization process has facilitated the gathering of such data by eliminating the wearisome task of searching for each food item on printed food-composition tables. However, the process of matching foods and portions recorded during assessment with those found in databases remains a difficult task.

Dietary analysis software programs are periodically updated, and new ones are often made available in the market. These attempt to include a more varied and updated array of new foods introduced into the market, and some also include common restaurant foods. Some software may be more appropriate than others depending on the needs of the dietitian and the context in which they will be used. Special considerations to take into account when choosing software are discussed in this section, as well as important default assumptions made about databases, analysis of new and ethnic foods, analysis of fluids and hydration status in athletes, and analysis of nutritional supplements.

1.4.1 INTERNET WEB SITES

The current availability of nutritional analysis tools includes Internet-based options that are free to the public. These can be used by dietitians as a sole nutrient database reference, as well as in combination with other software when foods from reports are not found in the currently used software. Although several free online nutritional-analysis software programs may be available, dietitians should consider their accuracy, especially when considering the use of free, Internet-based software. The U.S. Department of Agriculture (USDA) Food Composition Search Tool⁹⁴ and MyPyramid.gov⁹⁴ provide reliable information about caloric and nutritional content of foods based on scientific research conducted by the USDA.

The USDA Food Composition Search Tool can be accessed with the following link: <http://www.nal.usda.gov/fnic/foodcomp/search/index.html>.⁹⁴ This Web tool was created by the Nutrient Data Laboratory (NDL)⁹⁵ with the responsibility to develop the USDA's Nutrient Database for Standard Reference (NDSR), which is the basis of most food and nutrition databases in the United States, making this source a reliable option that is available to the public and scientific community. Nutritional analysis of a wide scope of foods can be obtained by entering up to five keywords to describe single food items, with an option to narrow the search by choosing the food group to which the specific food belongs and by choosing the amount of food to be analyzed. This search tool offers general food items as well as brand-name products and foods found in restaurant menus. It also offers the option of viewing reports on foods by single nutrients that are sorted either by food description or in descending order by nutrient content.

Moreover, the NDL homepage⁹⁵ offers accessibility to the Dietary Supplements Ingredient Database (DSID),⁹⁶ which was created by NDL researchers in conjunction with the Office of Dietary Supplements, the National Institutes of Health, and other federal agencies to estimate levels of ingredients in dietary supplement products. This tool could be especially helpful to analyze the intake of dietary supplements that might not be included in currently available dietary software. The DSID homepage⁹⁶ offers several features that dietitians can choose according to the context in which supplement intake analysis will be made. These include a research summary, data files, and a multivitamin/multimineral calculator for adults, with a basic and a professional version. More information is available for researchers. The use of the calculator allows for the gathering of estimates for specific nutrient levels listed on the Supplement Facts labels of some adult multivitamin/multimineral supplements. These estimates can aid dietitians in assessing total nutrient intake, since approximately one third of the population in the United States takes vitamin-mineral supplements daily.⁹⁷

Another reliable Web-based tool for nutritional analysis is MyPyramid.gov,⁹⁸ which was created by the Center for Nutrition Policy and Promotion branch of the USDA. This Web site offers several resources that are user-friendly and can be accessed by the public. One of these resources is MyPyramid Tracker.⁹⁸ MyPyramid Tracker is an online tool that can analyze nutritional and physical activity information. It has a Food Calories/Energy Balance feature that calculates energy balance, taking into account the information provided on foods eaten and physical activity performed, which is helpful to understand the energy-balance status of the person being analyzed. Energy-balance history can be saved and viewed in the system up to a year.

The dietary analysis portion of MyPyramid Tracker allows people to enter food items individually, using a similar format as mentioned for the NDL nutritional analysis tool, except that with MyPyramid Tracker all items consumed on a day can be then analyzed altogether, providing caloric and nutritional information of all foods consumed instead of analyzing a single item at the time. This Web tool offers the option of creating a list with frequently used foods to facilitate the process of entering foods for a given person after the first time.⁹⁸

Once all food items have been entered, the system analyzes food intake based upon the recommendations of the Dietary Guidelines for Americans, 2005. The system also uses information entered regarding age, gender, height, and weight of the person being analyzed. This information is then used to compare the actual food intake to the intake that would be recommended for the person. Comparisons are made according to the previously stated person's characteristics, the type and amount of physical activity performed, and the selection of weight maintenance or progressive weight loss chosen for the analyzed person.

Several analysis reports can be obtained from this system. They include nutrient intakes from foods, comparisons of intakes from basic food groups to the Dietary Guidelines for Americans, and comparisons between intake and the MyPyramid recommendations. In addition it is possible to obtain an assessment of intake over time (up to 1 year) with an average of MyPyramid recommendations by food group and nutrient intake for the days entered. This report includes graphs of daily intakes

with trend lines for each MyPyramid food group and individual nutrients to ease interpretation of results.⁹⁸

The energy-balance analysis provides information about total caloric intake, Estimated Energy Requirement (EER), percent of calorie intake from EER, and percent of calories expended from physical activity, and presents a graph of energy balance for a single day or multiple days (up to a year). This Web tool can be useful to dietetic students and dietitians who cannot afford or choose not to purchase other nutritional analysis software. It can be also used as a complementary source that has features, such as physical activity analysis, that may not be present in other software or databases. Also, since it is designed for the general population, this tool can be directly used by anyone interested in knowing about his or her nutritional and physical activity status.

It should be noted that the information provided in the NDL and DSID Web sites as well as in MyPyramid.gov has gathered data from foods (NDL and MyPyramid.gov) and nutritional supplements (DSID) available in the United States; therefore special considerations should be taken when analyzing diets of people consuming products that are not commonly available in the United States. Information regarding international nutrient-analysis databanks can be found at http://www.nutrient-dataconf.org/DatabankDir/IDB_Dir.htm.⁹⁹ This Web site offers an International Nutrient Databank Directory⁹⁹ that was prepared by a committee of the National Nutrient Databank Conference by compiling an updated international directory of software applications and their corresponding food composition databases. This directory can be useful for dietitians to establish the combination of sources that may best meet their needs and allow them to compare special software and database characteristics.

1.4.2 SPECIAL CONSIDERATIONS WHEN CHOOSING SOFTWARE

The increasing number of nutritional-analysis software packages available on the market poses a progressively more difficult task for dietitians and other potential users to decide which system would best suit their specific needs and objectives. These software range in price from free (Internet based) to more than \$10,000.¹⁰⁰ Price is an important consideration when choosing software. It should be noted that the price of the software differs in part on the availability of public funding or sponsoring agents. Thus, nonfunded manufactures develop software in a private manner, which tends to increase the cost of production and therefore the final cost of the product. Consequently, the price in itself does not necessarily reflect the quality of the final product.¹⁰¹ Another factor that affects the software's price is the number of users that it has; the more users, the more income developed and thus the lower the software price. Therefore, since generally there are more clinical than research users, software applications used by the former tend to be less costly than those used by the latter.¹⁰² Unique features present in more costly software geared to the scientific population might not be needed for counseling purposes; therefore, lower cost alternatives that better reflect the needs of the user should be considered.¹⁰¹

Although price may limit the decision to buy one type of software over another, this should not be the main focus when choosing nutritional-analysis software. Even within their price range, dietitians should aim to choose the software with the highest

quality of nutrient database as possible. Nutritional-analysis software reviews that are regularly published in journals such as the *Journal of the American Dietetic Association* and *Nutrition Today*¹⁰³ center on program features such as screen presentation, ease of entering foods and amounts, ability to modify the database, reporting capabilities, hardware requirements, and statistical-analysis functions. Even though these characteristics are important to consider at the time of choosing software, the quality of the nutrient database on which all calculations are based needs to be evaluated; otherwise the program features would be of little help if the nutrients calculated are of poor quality. Data quality refers to the suitability of the food values, ensuring that presented values are representative of the composition of the foods included on the database, and that the food items on the database are those consumed by the individual or population being analyzed.¹⁰¹

Many software programs provide demonstration packages that can be helpful for dietitians to test system features, such as data-entry and nutrient-calculation capabilities, before making a decision on which software to buy. However, decisions regarding the nutrient database component should not be based solely on these demonstrations, since foods and nutrient values included may not represent the complete database.¹⁰³ Several questions may arise when trying to determine the quality of the nutrient database of different systems. Buzzard et al. (1991)¹⁰³ proposed a series of specific questions to be used as guidelines in evaluating databases quality:

1. Does the nutrient database include all the foods and nutrients of interest?

Requirements about particular foods such as fast-food items, specific brand-name products, or less commonly consumed foods such as ethnic or vegetarian foods need to be identified before choosing a specific software, since there is a lot of variation regarding these characteristics among different software packages. Furthermore, there is variation related to food names, varieties of foods grown and distributed, usual recipes, and fortification rules and practices among different geographic regions within the United States and also between the United States and other countries. Therefore, dietitians should choose a database system that represents foods available to their research study or dietetic practice population.¹⁰¹

Also the number of food items listed on a database may not reflect the total capability of the software's system. Some software applications list a relatively small amount of foods; however, they can accommodate differences in food form, preparation, and amount units through their nutrient-calculation system, thereby allowing for an equal or more comprehensive number of food items than systems in which foods are entered separately in the database.

2. Do desirable food items contain complete nutrient analysis?

Nutritional-analysis software packages often present several numbers of missing values, leading to errors in the accuracy of nutrient calculations. Misinformation is usually not reported to buyers, and since missing values are listed as zeros, these could result in misinterpretation of the nutrient content of foods being analyzed. Dietitians should require vendors to provide them with an estimate of missing values for the nutrients included in the

database, thus allowing dietitians to check specific foods for completeness and currency of the nutrient data.¹⁰³

Moreover, dietitians can check detailed specifications available from the Standard Reference¹⁰⁴ and Food and Nutrient Database for Dietary Studies (FNDDS).¹⁰⁴ The Standard Reference only lists available values without providing zeros even when nutrients are known not to be present in a food (e.g., fat in table sugar), thus some food component fields are empty. On the other hand, in the FNDDS, all food component fields have a value, using zeros for absent nutrients or estimated values using imputations. Another source available to dietitians to check for the nutrient content of specific foods with missing values is the International Nutrient Databank Directory (which is sponsored by the National Nutrient Databank Conference). This directory is updated every 2–3 years and includes information on available food components for more than thirty databases.¹⁰²

3. Do the food descriptions incorporated in the database provide adequate specificity to accurately assess the desirable nutrients?

In order to answer this question without using the software, dietitians should consider the purpose and scope of their practice to determine whether information regarding special items such as low-sodium or nonfat versions of food products will be needed, whether they are available on the database of interest, and whether the software allows for the manual addition of specific items (although this would be a labor-intensive, tedious solution). An even more important factor to consider is the form in which manufactured foods are entered into the database. Data from manufacturers usually contain only nutrients from the food label or those required for nutrient claims or for educational purposes published by the company. Therefore when this source of food composition information is used in the database, there will be misinformation of certain nutrients for specific food items. Dietitians should be aware of this fact and should consult with the Standard Reference database to obtain a comprehensive nutritional analysis of specific food items with misinformation.¹⁰¹ Dietitians can request a listing of the descriptions of the foods included in the database and of their sources of nutrient content to evaluate the overall suitability of the software of interest.¹⁰³

4. What quality control procedures are used to guarantee the accuracy of the nutrient database?

Software users usually rely on the integrity of software developers to determine the accuracy of the nutrient database. Therefore quality control procedures may be required to ensure accuracy.¹⁰¹ Quality assurance procedures are required during the development of nutrient databases. They include a comparison of calculated algorithms with expected values for each database entry, cross-checking of all database changes, computerized edit checks to identify values that fall outside of specified ranges within each food group, and repeated calculation of test food records to guarantee that any differences between database versions are due to intended modifications to the software and not due to error.¹⁰³

Answering these questions will provide the foundation for a comprehensive review and evaluation of the nutrient database. However, the final selection of the most suitable software package will depend on the additional system features that best reflect the particular needs of the dietitian. Important features to aid in the selection of software are the system's cost and the type of output generated by the system.

The type of output chosen differs based upon the specific needs of the user. For example, for an intervention study, the composition of foods consumed over a prolonged period would be the chief information needed, while a menu developer may find the composition of foods intended for a meal to be the most valuable information. In general, dietitians should choose software that summarizes nutritional information analyzed by each meal or menu, or as an average of meals consumed during one or several days, depending on which analysis would be most helpful to them.¹⁰¹

In some situations, dietitians may need to print materials (for example, for educational purposes), in which case the software chosen should be able to provide simple and concise outputs. The availability of printed graphs, for instance, would be very useful to demonstrate adequacy of nutrients to meet specific aims during counseling sessions or interventions.¹⁰¹ On the other hand, for educational purposes involving overall nutrition, displays showing food groups instead of individual components may be more suitable. For instance, MyPyramid Tracker⁹⁸ offers the option of comparing the intake of an individual with the MyPyramid recommendations, which could serve as an evaluation instrument. Yet for research purposes, results may need to be exported to a spreadsheet format to assist in the creation of summaries, and in the performance of statistical analysis for comparison between different set of data.¹⁰¹

Choosing software that best fits the needs of the dietitian is not an easy task. A more detailed set of guidelines to further assist professionals in choosing the most suitable software can be found in [Table 1.4](#).^{103,105–107} The appropriate selection of software would enhance the accuracy and utility of the dietary assessment process as a whole.

1.4.3 DATABASES

Determining the appropriate composition of nutrients in foods is of great importance. Food composition databases can be used to plan and evaluate dietary competence of meals and overall diets. However, accurate assessment of the diet can only be achieved with an accurate database.

There are other considerations when deciding on which database to use. Recognition of the target audience, efficiency of the search system for obtaining nutrient data, the content and format of summary information, and cost are all important considerations. With increasing use of the Internet, there are a few freely accessible online databases that are used by clinicians, athletes, the general public, and researchers. There is also software that can be purchased for more specific uses. Costs of this software vary from less than US\$100 to as much as US\$8,000. The more expensive systems contain more unique and customized features.¹⁰⁸ Lists of software can be found in the International Databank Directory.

TABLE 1.4
Guidelines to Choose Dietary Software

Software-computer compatibility	<ul style="list-style-type: none">• Check RAM, hard disk space, memory configuration, operating system, monitor, and printer requirements
Type of license needed	<ul style="list-style-type: none">• Check whether you need single-user or multiple-user license
Software documentation	<ul style="list-style-type: none">• Check documentation to: install and manage software; run all the software features; troubleshoot; manage files• Check whether documentation specifies default assumptions
Type of database	<ul style="list-style-type: none">• Check source of information for nutrients; recency of data; availability of database upgrades; notification of database updates; accessibility to all nutrients of interest• Check whether foods with missing data are flagged and whether the totals including these foods are also flagged
Fluid consumption	<ul style="list-style-type: none">• Check whether software allows you to enter water/fluid consumption or whether it contains water content of foods that can be adjusted to record fluid consumption
Addition of new foods/recipes	<ul style="list-style-type: none">• Check whether software allows you to add new foods/recipes to the database, the ease with which to do so, the maximum amount of new foods/recipes that can be added, and whether the recipe system includes a retention (cooking gains/losses) algorithm
Type of output produced	<ul style="list-style-type: none">• Check whether software output will satisfy your needs• Check whether percent of energy from macronutrients is provided; whether intakes are broken down by meal; whether intakes are assessed relative to standards and if so, whether the standards are current and accurate; whether weight management algorithms are included, and if so, whether they are suitable for athletes
File management	<ul style="list-style-type: none">• Check for ease of file management use• Check whether software generates multiple-day averages for individuals• Check whether data produced is compatible with statistical software for research purposes

Sources: Data from Sugerman, S.B., Eissenstat, B., and Srinith, U., Dietary assessment for cardiovascular disease risk determination and treatment, in *Cardiovascular Nutrition. Strategies and Tools for Disease Management and Prevention*, Kris-Etherton, P. and Burns, J.H., Eds., American Dietetic Association, Chicago, 1998, pp. 39–71; Buzzard, I.M., Price, K.S., and Warren, R.A., Considerations for selecting nutrient-calculation software: Evaluation of the nutrient database, *Am. J. Clin. Nutr.* 54, 7–9, 1991; Grossbauer S., The number game, *Byting In*, 7, 3, 1997; Sugerman, S., What makes a software package worth buying? *Byting In*, 7, 1, 1996.

Currently, one of the most widely used databases for the United States is the Nutrient Database for Standard Reference (NDSR), which is maintained by the USDA. This database is freely available and includes around 7,293 foods and about 140 nutrients for each food.¹⁰⁹ All data on food and nutrient composition in the NDSR are developed from the Agricultural Research Service (ARS) food analysis, the food industry, scientific literature, and some are estimated and calculated based on recipes. It is important to mention that there are missing nutrient values for some

of the foods in the NDSR database, owing to the fact that some foods have not yet been estimated.

An additional database upheld by the USDA is the Food and Nutrient Database for Dietary Studies (FNDDS). This is used primarily to assess dietary intakes for the National Health and Nutrition Examination Survey. It contains about 7,000 foods and sixty-two nutrients for each food. Nutrient information in this database is obtained from the NDSR. In contrast to the NDSR, however, the FNDDS contains all the missing values on nutrient composition and therefore makes the database complete for computation of nutrient assessments.

Currently, the Office of Dietary Supplements at the National Institutes of Health is working on producing a nutrient database specific to dietary supplements. With such modest information on nutrient composition of supplements available and such growing use of various supplements, it is necessary to include this information to obtain an accurate overall nutrient analysis.¹¹⁰ New research on nutrient and non-nutrient components of food is uncovering items that are beneficial to health and aid in the prevention of disease. Because of this, expanding the database to include these components is an important goal and will allow greater accuracy when recording and estimating athletes' or patients' nutrient consumption and relative health risks. Most databases are, however, routinely updated to stay consistent and ensure quality and accuracy.¹¹¹ MyPyramid.gov is another freely accessible database with a Web site that allows for the assessment of dietary intakes based on food groups (fruits, vegetables, grains, dairy, fats, and protein). Once these intakes are determined, they are able to be compared to the MyPyramid recommendations and can then be incorporated into analyses for disease risk.⁹⁸

Although dietary databases are kept up to date and try to incorporate vitamins and minerals as well as nonnutrient substances, it is difficult to ascertain the actual intake of the athlete due to the variability of nutrients in similar foods. Variability accounts for inherent, environmental, processing, and analytical factors. Nutrient variability needs to be an important consideration when analyzing data. The dietitian must analyze possible differences in food variability and know any major differences between different foods that may have significant importance to the athlete or patient.¹⁰⁹

1.4.4 RECIPES

1.4.4.1 New Foods

The food supply is ever changing, and therefore the addition of new foods to the diet may cause challenges in maintaining a database. Since 2004, over 1,900 new food products have been manufactured from the top twenty-five food manufacturers alone. When these new foods are introduced into the market, it is the job of the database manager to determine if the food should be added and if the product will be in demand by consumers. Since 2005 and the changes in the dietary guidelines on consumption of whole-grain carbohydrates, databases have added several whole-grain food products to their systems because of such high demands by the consumer. There was an increase in the percentage of consumed whole-grain breads (12%),

whole-grain rice (19%), and whole-grain ready-to-eat cereals (16%).¹¹² Additionally, databases must also distinguish between the various brands and their different nutrient components to ensure accurate analysis.

Although several measures are being taken to keep databases updated, users often find that there are some foods not in a system. When these foods are omitted but are a main diet staple of the user, it makes it difficult to conduct a proper dietary analysis. The USDA Web site does allow one to inform database manufacturers about the need for additions. Food labels on products are another way to obtain some nutrient information about a food product that may not be available on the database. However, food labels are often very incomplete and do not offer total nutrient composition. If more information on nutrient composition is warranted, there is usually a phone number on the product to call for further information.

There are a few software database systems that allow the dietitian to add new foods and their nutrient content to the current listing of foods. This feature can be beneficial with athletes since they often consume foods that are not found among database food choices, such as sports bars, shakes, and energy products.

1.4.4.2 Default Assumptions

Recipes or combination foods constitute another issue for accurately determining nutrient intake. Although some databases and software (such as FNDDS) contain combination foods, like lasagna, preparation and ingredients often vary. Similarly, foods such as fried chicken are found in database systems and the listed ingredients assume it is made a certain way. If the chicken consumed is fried differently than the software assumes, there may be significant differences in nutrient analysis. If this is the case, analysis may be erroneous due to the variability of food components. Therefore, combination foods may be best analyzed by adding the ingredients and ingredient amounts separately. Although it may take more time, it will ensure a more precise analysis of nutrient intake.

If eating out, as athletes often do while traveling, many restaurant Web sites now have nutrient information listed on all of their dishes and are easily accessible. In software, just as addition of new foods to listings is available, addition of combination foods and recipes is also available. In the case of training tables for athletes, the chef should be able to provide the nutrient information from foods to the dietitian and allow for a precise nutrient analysis and addition into a software database.

1.4.4.3 Ethnic Foods

The International Food Composition Tables Directory¹¹³ is a collection maintained by the United Nations International Network of Food Data Systems. It provides lists (electronically and hardcopy) of databases from around the world. It can be used to aid in locating and analyzing data on ethnic and imported foods that are not available in U.S. databases. This is beneficial to athletes during times of travel to other countries and when consumption of ethnic foods is a factor.¹⁰⁹

Recent globalization of the food supply has the ability to alter accurate nutrient analysis. Even though it may be the same fruit or vegetable, growing conditions and storage must be considered. Databases have one common nutrient analysis for each

fruit or vegetable, and only if the nutrient content is significantly different will additions be made to the database. This has been the case with various potato varieties (such as purple and yellow heritage potatoes), which vary in their carotenoid and flavonoid content from a regular potato.¹⁰⁹

1.4.5 FLUIDS AND HYDRATION

Water makes up 50–60% of our body weight. Water is essential for many of our body functions, including heat regulation, participates in chemical reactions, and is used as lubrication, as a transport medium, and as a solvent during ionization of electrolytes and acids. Body water is found most abundantly in the skin, organs, muscle, and blood. Thus, hydration by water as well as other beverages before, during, and after physical activity is essential to maintain a healthy lifestyle and peak performance. This section addresses fluid replacement and the role of water and sports drinks in hydration as well as the influence of hydration on physical performance.

The American College of Sports Medicine's position stand for exercise and fluid replacement states the following: (1) prehydration is essential and should begin several hours prior to exercise, (2) develop a plan for fluid replacement during exercise that will prevent more than 2% dehydration, (3) fluid held at a temperature between 15 and 20°C will be preferred over warmer beverages, (4) consume fluids containing 20–30 milliequivalents per liter of sodium, 2–5 milliequivalents per liter of potassium, and 6–8% of carbohydrates to help sustain electrolyte balance and exercise performance, and (5) consuming beverages with meals postexercise will expedite rapid and complete recovery due to the stimulation of thirst and fluid retention.¹¹⁴

1.4.5.1 Sports Drinks

When sports drinks were developed, they changed the way athletes hydrated before, during, and after events. Sports drinks are made up of three main components: water, carbohydrates, and electrolytes.¹¹⁵ Carbohydrates and electrolyte replacement, in addition to water replacement, is important to assist the body in recovery, especially after prolonged exercise as well as performing in a hot, humid environment. [Table 1.5](#) lists some popular sports drinks and their relation to other beverages, such as water, orange juice, and diet and regular soft drinks. As discussed previously, it is important to understand what an individual is consuming prior to, during, and after exercise to determine if he or she is adequately hydrating and replenishing fluid stores.

The form and concentration of carbohydrates contained in drinks have been studied extensively to determine which would assist in performance and replenish stores. Some carbohydrates have led to gastrointestinal upset and as such may not be a good recommendation for athletes. However, since every individual is different, it is important to try different sports drinks during training to determine which, if any, work best for the individual. Gisolfi and colleagues (1998)¹¹⁷ used different formulas of carbohydrates to determine if the beverage osmolality would have any effect on gastric emptying and thus affect water availability. Each beverage contained 6% carbohydrates but with different formulas. The carbohydrate beverages were not different in comparison to gastric emptying; however, the water that was consumed in the control group was absorbed faster.¹¹⁷

TABLE 1.5
Carbohydrate-Electrolytes Beverages versus Water

Beverage	Carbohydrate Ingredient	Carbohydrate (% concentration)	Grams of Carbohydrate	Sodium (mg)	Potassium (mg)
Gatorade Thirst Quencher (Gatorade Company)	Sucrose, glucose, fructose	6	14	110	25
Gatorade Endurance Formula	Sucrose, glucose, fructose	6	14	200	90
PowerAde (Coca-Cola Company)	High-fructose corn syrup, maltodextrin	8	19	55	30
All Sport (Monarch Beverages)	High-fructose corn syrup	9	21	55	55
Coca-Cola	High-fructose corn syrup, sucrose	11	26	9.2	Trace
Diet Soft Drinks	None	0	0	0–25	Low
Orange Juice	Fructose, sucrose	11	26	2.7	510
Water	None	0	0	Low	Low
Gatorade Energy Drink (Gatorade Company)	Maltodextrin, glucose, fructose	23	53	133	70

Source: Modified from Williams, M.H., *Nutrition for Health, Fitness, and Sport*, 8th ed., McGraw-Hill, New York, 2007.

1.4.5.2 Water

As discussed above, water is able to be absorbed quicker than the 6% sports drinks, resulting in more rapid replenishment, which would be vital during exercise under certain conditions. Ryan and colleagues (1998)¹¹⁸ also found that consuming water during exercise, even when an individual is hypohydrated, had a slightly higher absorption than different carbohydrate drinks.¹¹⁸ However, there are many different aspects that can affect gastric emptying. The main aspects are exercise intensity, the pH of the stomach, mode of exercise, volume of fluid, caloric density, drink temperature, electrolytes, heat stress, and osmolality. Since water plays a vital role within the body, it is important to replenish fluid stores and to stay adequately hydrated. Therefore, when considering hydration for training and performance, water is a cheap and easy option, and it should not be dismissed in preference to sports drinks.

1.4.5.3 Other Beverages

As seen in [Table 1.5](#), other beverages have been used to assist in hydration. Soft drinks are one option that athletes may choose due to their high carbohydrate content. This choice is often a matter of taste preference, however. It should be noted that many soft drinks contain caffeine, which is considered a diuretic and may negatively affect hydration status. Likewise alcoholic beverages are inadvisable for the same reason.¹¹⁹ Fruit juices, on the other hand, not only provide a good source of water but also may have higher content of carbohydrates. This may be advantageous for some individuals; however, juice consumption is very individualized and care must be taken since some individuals may have gastrointestinal upset due to lower pH of the juices.

1.4.5.4 Fluid Hydration Status

Hydration status may be categorized one of four ways. First, dehydration results when the body fluid volume is decreased. The term *hypohydration* is also used in similar situations as dehydration and is defined as the rate of fluid intake that is less than the rate of fluid loss. Dehydration or hypohydration has been shown to impair exercise performance and can lead to detrimental effects on the individual if not corrected. Voluntary dehydration, which has been used by some athletes to qualify for a lower weight class, could have a possible detrimental effect on performance. Research clearly shows that voluntary dehydration does not improve performance and may affect cognitive functioning.¹²⁰ Involuntary dehydration occurs most often during prolonged aerobic activities and can have a major effect on performance and, more importantly, on health. When individuals become dehydrated, cardiovascular functions and temperature regulation often become compromised and physical performance may be adversely affected¹¹⁴; as seen in more severe cases, an athlete may have a reduced sweat rate¹²¹ and heat illness may result.

Second, hyperhydration reflects a status where the rate of fluid intake is greater than the rate of fluid loss. This can be helpful in regulating body temperature and cardiovascular functions when the rate of fluid intake during performance cannot keep pace with the rate lost during exercise.¹²² While there is no evidence to suggest

that hyperhydration improves performance *per se*, hyperhydration prior to some distance events performed in hot, humid environments may minimize performance decrements. Therefore, the American College of Sports Medicine recommends that hyperhydration be used before exercise or performance in heat environments.⁶¹ Cold water or a glucose–electrolyte solution can assist athletes in hyperhydrating.¹¹⁶

Euhydration is defined as the rate of fluid intake that is adequate to replace fluid losses. It is important for athletes to be euhydrated or, in certain circumstances, hyperhydrated prior to exercise or an event. Being properly hydrated helps in minimizing fluid loss and performance, and may prevent heat-related illness.¹¹⁶

Rehydration results when an individual consumes fluids in an effort to replenish fluid lost during an event that has caused the body to be in a dehydrated state. Rehydrating can also reduce the rise in body temperature and minimize the stress on the cardiovascular system during longer periods of endurance exercise.¹¹⁶

It is critical for athletes to rehydrate following exercise training or competition to ensure proper performance during the next day's training or event. As mentioned in [Section 1.3](#), rehydration is needed and can be simple using the correct techniques. Being aware of the amount of time available for rehydration is imperative. It is important to weigh prior to exercise and then immediately after. By comparing these numbers, rehydration can be determined. For each kilogram lost, a person should consume 1.5 liters of fluid if needing to rehydrate in a short period of time, approximately 12 hours.¹²³ Plain water with food that contains sodium to assist in the replacement of electrolytes will be able to adequately rehydrate an athlete who has an extended amount of time for rehydration.

1.4.6 SUPPLEMENT ANALYSIS

Analysis of supplements will assist in the nutritional diagnosis of an individual. [Table 1.6](#) contains a list of some of the most common supplements in use. However, due to the wide array of supplements on the market as well as continual production of new supplements, this list is not meant to be all-encompassing. Nevertheless, it is important to read the supplement labels as well as view the recent research and Web sites of the governing bodies to determine if the supplement is not only effective but more importantly both safe and acceptable for use. As a reminder, because supplements are not regulated by the Food and Drug Administration, it is imperative to research and review the supplement prior to recommending it for use.

1.5 CONCLUSIONS

In this chapter the estimation of food and nutrient intake of athletes has been reviewed, concentrating on three main aspects: (1) methods of assessing food intake, (2) special issues with assessing food intake in athletes, and (3) translation of dietary assessment into analysis.

There is a variety of methods of assessing dietary intake, such as diet records, 24-hour dietary recalls, and food frequency questionnaires. When conducting dietary assessments of individuals, it is important to be aware of certain issues,

TABLE 1.6
Supplements: Their Safety and Effectiveness

Supplement	Safety (at Recommended Doses)	Effectiveness
Androstenedione	Safety concerns about chronic use	Not effective
Branched chain amino acids	Possibly safe	Not effective to delay fatigue; some studies found related immune system support
Caffeine	Possibly safe; known to have adverse effects that could affect performance	Effective as a stimulant to the central nervous system
Carnitine	Possibly safe	Results from studies are mixed in terms of effectiveness
Chromium picolinate	Safety concerns with chronic use	Not shown to be effective for increasing muscle mass as well as decreasing fat mass
Conjugated linoleic acid	Possibly safe	Results from studies are mixed in terms of effectiveness
Creatine	Possibly safe	Effective in increasing lean body mass in weightlifters and high-intensity, short-duration performance
Dehydroepiandrosterone (DHEA)	Safety concerns with chronic use and acute high doses	Not effective
Glucosamine/chondroitin sulfate	Possibly safe	Effective in some individuals
Glutamine	Possibly safe	Results from studies are mixed in terms of effectiveness
Beta-hydroxy-beta-methylbutyrate (HMB)	Possibly safe	Results from studies are mixed in terms of effectiveness
Medium-chain triglycerides	Safety concerns with acute and chronic use	Not effective
Multivitamin and mineral supplements	Safety concerns with doses, in conjunction with the diet, that would exceed the upper intake level (UL)	Effective with nutrient deficiencies; daily multivitamin use is recommended by some to prevent chronic disease
Protein	Possibly safe for individuals without known or unknown kidney or liver disease	No difference in comparison to food protein
Pyruvate	Possibly safe	Not effective
Ribose	Possibly safe	Not effective

Source: Modified from Dunford, M., *Sports Nutrition: A Practice Manual for Professionals*, American Dietetic Association, Chicago, 2006, p. 131.

paying particular attention to misreporting, snacking, and openness in reporting. A successful dietary assessment depends on the expertise of the registered dietitian, who needs to be aware of the client's perceptions toward food as well as toward the professional. Special issues that sports dietitians need to be familiar with regarding

nutritional assessment of the athletic population include periodization, fluid intake, supplements, and traveling. In order to effectively translate dietary assessment into analysis, sports dietitians should evaluate the population they are working with, as well as the cost and quality of the extremely wide availability of software and databases in the market. They should also be aware of several factors such as ethnic foods, fluid replacement beverages, and supplements that may not be included in databases. Therefore, a very detailed dietary assessment needs to be performed in order to conduct the necessary research that would in turn translate into a more accurate dietary analysis and future recommendations.

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2 Evaluation of Nutrient Adequacy of Athletes' Diets

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2.1 INTRODUCTION

In today's competitive sports environment, athletes need to be physically and mentally fit to perform at their best. Research clearly shows that nutrition can play an important role in improving exercise performance, decreasing recovery time from strenuous exercise, preventing exercise-associated injuries due to fatigue, providing the fuel required during times of high-intensity training, and controlling weight.¹ Adequate and proper nutrition is important for active individuals to meet their overall energy, nutrient, and fluid needs. Thus, many athletes are interested in learning how to improve their dietary and fluid intakes for health and performance. One of the first steps in determining how to best improve an athlete's diet is to assess his or her food, fluid, and supplement intakes within the context of their weight goals, sport training routine, and competition schedule. Knowing when an athlete eats in relationship to exercise training may be as important as knowing what he or she eats. Regular assessment of

an athlete's diet will help identify potential nutrition problems related to time of year, changes in training routine, health issues that arise such as injuries or illness, and/or lifestyle changes. This chapter reviews the methods used to assess an athlete's diet and the guidelines used to determine the adequacy of these diets, including the dietary reference intakes, approaches for assessing dietary adequacy, and specific macro- and micronutrient recommendations for active individuals and athletes.

2.2 DIETARY ASSESSMENT

There are a number of traditional dietary assessment methods such as diet histories, food records, diet recalls, and food frequency questionnaires that can be used to estimate dietary intake patterns and nutrient intakes of individuals or groups of athletes.² These diet assessment tools are discussed in detail elsewhere.²⁻⁴ However, it is important to understand the errors associated with each of these methods and how to use each method to provide the best picture of an athlete's or active individual's typical dietary pattern for each phase of the training program. The more accurate these dietary assessments can be, the more likely it is the sports dietitian will obtain the data needed to help the athlete achieve his or her performance, weight, or health goals. Research shows that the accuracy of self-reported dietary intake data is influenced by a number of factors such as age, gender, body weight and composition, restrained eating behaviors, socioeconomic status, and cultural influences.⁵ This reporting bias can lead to misinterpretations of the energy and nutrient adequacy of an individual's diet and nutritional status.⁶ Depending on the instrument used and the individual assessed, self-reported food intake can be under- or overestimated, which will result in misinterpretation of an individual's energy and nutrient intakes.^{5,7} Based on research comparing doubly-labeled water (DLW) as a measure of energy expenditure to various dietary assessment methods assessing energy intake, individuals are most likely to underreport energy intake.⁷ Underreporting of food intake, and thus energy intake, can occur for a number of reasons, such as underreporting portion size, forgetting foods, not recording alcohol and high-energy snack foods, changing eating behaviors to avoid eating foods that are hard to record, or eating healthier foods during the recording period. Additionally, length of the dietary evaluation period also needs to be given attention because of day-to-day variations in energy intake due to food availability, training schedules, and time constraints. It is thus best to evaluate macro- and micronutrient contribution of the diets of athletes over several days rather than looking at a single day's intake. It is also best to examine the diet during different phases of the training program, since diet can change as the exercise training program changes. Thus, it is not only important to use the appropriate dietary assessment method for the individual or group but to also train the individual to report food and fluid intake as accurately as possible.

2.3 DIETARY GUIDELINES

How do we know we are eating the right foods for good health and performance? Do athletes have different nutrient needs than sedentary individuals? How do nutritionists and dietitians know what type of dietary recommendations to

make to athletes and active individuals? Nutritionists use a number of resources to make dietary recommendations to both groups and individuals. Two national evidence-based dietary guidelines frequently used are the Dietary Guidelines for Americans and the Dietary Reference Intakes. Dietary Guidelines for Americans are introduced next, while the Dietary Reference Intakes are discussed in the following section.

The Dietary Guidelines for Americans (2005) are developed by the U.S. Department of Agriculture (USDA) and the Department of Health and Human Services (DHHS) (<http://www.cnpp.usda.gov/DGAs2005Guidelines.htm>) to guide dietary recommendations for populations and individuals. This evidence-based document, which is revised every 5 years, is the cornerstone for U.S. nutrition policy and nutrition education activities. To help consumers evaluate their diet and make better eating choices, the Dietary Guidelines were used to develop MyPyramid and associated nutrition and diet tools (<http://www.cnpp.usda.gov>). The 2010 Dietary Guidelines for Americans are currently under development (<http://www.cnpp.usda.gov/dietaryguidelines.htm>).

The 2005 Dietary Guidelines for Americans were designed to convey dietary recommendations and not medical advice, so it is the job of nutrition professionals to help their clients interpret and implement these guidelines into practical eating behaviors, attitudes, and healthy lifestyle changes. The nine key recommendations of the 2005 Dietary Guidelines for the general population are listed below:

1. Consume a variety of foods within and among the basic food groups while staying within energy needs.
2. Control calorie intake to manage body weight.
3. Be physically active every day.
4. Increase daily intake of fruits and vegetables, whole grains, and nonfat or low-fat milk and milk products.
5. Choose fats wisely for good health.
6. Choose carbohydrates wisely for good health.
7. Choose and prepare foods with little salt.
8. If you drink alcoholic beverages, do so in moderation.
9. Keep food safe to eat.

Similar documents have been developed in other countries. For example, Canadians use Canada's Guidelines for Healthy Eating (Health and Welfare Canada 2007) and the Canada's Food Guide to Healthy Eating. Australia (Dietary Guidelines for All Australians, 2003) (<http://www.nhmrc.gov.au/PUBLICATIONS/synopses/dietsyn.htm>) and New Zealand (Food and Nutrition Guidelines) (<http://www.moh.govt.nz/foodandnutrition>) also have similar documents. In Switzerland, the Society for Nutrition (Schweizerische Gesellschaft für Ernährung) developed the Swiss Food Guide Pyramid. This document was used to design a food pyramid geared toward active individuals and athletes (www.sfsn.ch).⁸

2.4 DIETARY REFERENCE INTAKES

Over the past 10 years, the Institute of Medicine (IOM) Food and Nutrition Board (FNB) of the National Academy of Sciences has revised the energy and nutrient recommendations for Canadian and U.S. populations. These new recommendations reflect the growing body of scientific evidence that chronic diseases may alter nutrient requirements. These recommendations are termed the Dietary Reference Intakes (DRIs), which reflect a family of reference values. These values are designed to prevent nutrient deficiency and reduce the risk of chronic diseases for the population in general but also to provide valuable guidelines when working with active individuals.

The various DRI reference values include the following: the Recommended Dietary Allowance (RDA), the Adequate Intake (AI), the Estimated Average Requirement (EAR), the Tolerable Upper Intake Level (UL), and the Acceptable Macronutrient Distribution Ranges (AMDRs). Each of these values is briefly explained below. A more detailed explanation of the DRIs for micro- and macronutrients, water, and electrolytes can be found elsewhere.^{9–14}

- **Recommended Dietary Allowance (RDA):** The RDA is considered the nutrient intake that meets the requirement of almost all (97–98%) of the healthy individuals in a specific age and gender group. Based on the scientific evidence available at the time, the DRI committees calculated RDAs for vitamin A, vitamin C, vitamin E, phosphorus, magnesium, copper, iron, iodine, molybdenum, selenium, zinc, thiamin, riboflavin, niacin, vitamin B₆, folate, and vitamin B₁₂.^{9–12}
- **Adequate Intake (AI):** When scientific evidence was not sufficient to determine an RDA, an AI value was calculated from experimental or observed intake levels that appear to sustain a desired indicator of health. AIs can be used as a goal for intake where no RDAs exist. The DRI committees have set AIs for vitamin D, vitamin K, fluoride, pantothenic acid, biotin, choline, calcium, chromium, manganese, potassium, sodium, and chloride.^{9,10,12,13}
- **Estimated Average Requirement (EAR):** In order to determine an RDA, the nutrient intake value estimated to meet the requirement of half the individuals in a specific group. This figure is used as a basis for developing the RDA. For example, the RDA for a particular nutrient is calculated as follows: $RDA = EAR + 2 SD_{EAR}$ where SD_{EAR} is the standard deviation of the EAR. If data about the variability in requirements are insufficient to calculate a standard deviation, a coefficient of variation (CV) for the EAR of 10% is ordinarily assumed.
- **Tolerable Upper Intake Level (UL):** The UL is the maximum intake of a nutrient by an individual that is unlikely to pose a risk of adverse health effects to most healthy individuals. If intakes of a nutrient are above the UL for an extended period of time, the risk of adverse effects increases. The UL typically refers to total nutrient intake from food, fortified foods, and supplements. The term “tolerable intake” was chosen to avoid implying a possible beneficial effect from this level of the nutrient. However, some

ULs were set with limited information and may change in the future as new information becomes available.

- ***Acceptable Macronutrient Distribution Ranges (AMDRs):*** AMDRs have been established for fat, protein, and carbohydrate. Carbohydrate also has a minimum recommended amount, and protein has an established RDA value based on age and gender. Water, total fiber, and essential fatty acids have established AI values.^{13,14} The total water recommendation includes all water contained in food, beverages, and drinking water.

2.5 APPROACHES TO ASSESSING DIETARY ADEQUACY

In addition to the aforementioned national guidelines, there are a number of other resources sports dietitians can use to make dietary, fluid, and supplementation recommendations to active individuals and athletes. These specific recommendations, frequently in the form of position papers, consensus statements, and sport-specific recommendations, synthesize the current science-based information and translate this into guidelines for athletes.^{15–22} In [Sections 2.5](#) and [2.6](#), we integrate these more specific dietary recommendations for athletes within the context of the Dietary Guidelines for Americans and the DRIs.

In order to assess dietary adequacy of athletes training and competing in sports, it is essential to examine the energy demands of the various activities comprising the training program. Training and competition vary considerably in many sports. While training may challenge the athlete in terms of maintaining energy balance and nutrient stores through the preparatory months, during competition many sports focus solely on racing interspersed with conditioning to keep athletes fit. Thus, evaluating dietary adequacy must take training and competition into account. In addition, most training plans are organized in phases with varied volume and intensity and progressing from general to sports-specific training. Coaches typically periodize training programs, which means that training and rest are balanced carefully in order to maximize the athlete's training adaptation. Assessment methods should be used that help differentiate various training phases and recovery periods. This can be done by a variety of approaches.

To evaluate whether an athlete eats adequately (appropriate quantity, quality, and timing of energy and nutrient intake), the energy demands of the sport must be known. To assess energy expenditure in athletes at the least complex level, the sports dietitian can select physical activity levels (PAL) or physical activity coefficients (PA) in calculations deriving energy expenditure requirement (EER; DRIs energy) and/or multiples of resting metabolic rate (RMR). RMR can be measured or assessed. Thompson and Manore²³ have shown that the Cunningham²⁴ equation is best suited for use in male and female endurance athletes. Other quick approaches include reference tables summarizing energy cost for a given person relative to weight and sport.¹ More time consuming, burdensome, and complex are assessment techniques that involve physical activity records using metabolic equivalents for 24 hours²⁵ or less (e.g., detailing exercise energy expenditure, EEE) and physical activity protocols.²⁶

TABLE 2.1
Energy Expenditure in Various Sports Using Doubly-Labeled Water (DLW)
Compared to Energy Intake Estimated by Dietary Recall

Sport	Energy Expenditure (kcal·d ⁻¹)	Energy Intake (kcal·d ⁻¹)	Reference
Runners	3492 (F)	2318 (F)	27
Runners	2990 (F)	2039 (F)	28
Tour de France	8604 (M)	6214 (M)	29
Lightweight rowers	3957 (F)	2214 (F)	30
Cross-country skiers	4373 (F)	4350 (F)	31
	7217 (M)	7217 (M)	
Gymnasts	1987 (F/M)	1745 (F/M)	32
Speed skaters	4015 (M)	not assessed	33

Note: F = females; M = males.

The first step, however, when evaluating the energy demand of a particular sport is to search the research literature. It is possible that studies have been conducted using DLW (see Table 2.1) or other more valid and reliable techniques than recalls.

In addition, some sports have advanced in technology and offer ways to quantify total work accomplished. One such example is the sport of cycling in which it is not uncommon that athletes use power meters integrated into pedals and crank arms. Martin et al.³⁴ conducted a study in cyclists using a power meter to quantify EEE. Data from such studies along with a more individualized or team assessment should be sufficient to identify target energy expenditure and macronutrient ranges for a thorough evaluation of energy and nutrient adequacy in athletes training and competing in a particular sport.

In addition to understanding the annual training and competition plan when evaluating dietary adequacy in athletes, the individual’s body weight and composition goals must be known. For athletes needing to lose body weight and fat, the sports dietitian must ensure minimal nutritional risk for energy and for macro- and micro-nutrients. Nutritional risk for athletes is different than for nonathletes. A diet reduced in macronutrients, and thus calories, could pose minimal risk to a nonathlete, while for the athlete energy intake (EI) is simply too low to meet all physiologic functions beyond what is necessary for exercise. This concept is referred to as energy availability (EA) and is derived from EEE subtracted from EI.¹⁸ For female athletes in particular, reducing EI while continuing with hard training poses a risk due to the link between low EA and menstrual dysfunction. Menstrual dysfunction can result in compromised bone mass as part of the female athlete triad.³⁵ Further, low EA can lead to glycogen depletion, micronutrient deficiencies, and fatigue. Manore et al.³⁶ proposed guidelines for maintaining energy availability in exercising women during various phases of weight loss, maintenance, growth, and recovery (see Table 2.2). These values can be used to evaluate a female athlete’s energy intake relative to various phases of training, competition, and growth.

TABLE 2.2**Recommended Levels of Energy Availability for Female Athletes during Various Phases of Training, Competition, Growth, and Weight Maintenance**

	Weight Loss (kcal·kgFFM ⁻¹ ·d ⁻¹)	Maintenance (kcal·kgFFM ⁻¹ ·d ⁻¹)	Growth/Intense Training/Racing (kcal·kgFFM ⁻¹ ·d ⁻¹)
Energy Availability	30–45	45	>45

Note: FFM, fat-free mass.

Source: Manore, M.M., Kam, L.C., and Loucks, A.B., The female athlete triad: Components, nutrition issues, and health consequences, *J. Sports Sci.* 25 Suppl 1, S61–S71, 2007.

With regard to carbohydrate (CHO) consumption, reduced intakes ($< 5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) in individuals exercising for the purpose of weight control and fitness are not problematic. For the athlete, however, CHO adequacy is essential for the maintenance of glycogen stores and the ability to recover from training on a daily basis.^{37,38} Depleting glycogen stores during heavy training may pose both performance and health risks to the athlete. A minimal CHO intake level of $5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ is considered necessary to maintain glycogen stores during intense training.³⁷ Obviously, weight loss and body composition manipulations should be attempted during off-season and low-intensity training phases. However, this is not always possible nor is it practical. Therefore utmost attention should be paid to CHO adequacy. While dietary assessment methods (see Chapter 1) can help evaluate CHO intake, performance indices along with subjective ratings of fatigue, mood state, and hours of sleep in combination with interdisciplinary approaches to monitor performance and health in the athlete can help evaluate overall stress and risk for underrecovery and overtraining³⁹ related to CHO inadequacy.

Energy restriction can also affect other nutrients such as protein and micronutrients and thereby increase the risk of unwanted side effects such as lean tissue loss and vitamin/mineral deficiencies, respectively. Protein intake needs to be monitored during phases of energy restriction, especially if strength and power should not be compromised. Higher protein intakes may be needed to preserve muscle mass during phases of weight and fat loss,⁴⁰ and micronutrient intake may be low, especially if EI falls below 1800 to 2000 kcal per day.

Vitamin and mineral recommendations are not based on activity level; thus, the DRIs can be used to assess adequacy. Further, many high-level athletes integrate routine biochemical testing for the purpose of screening and monitoring for health and performance. Biochemical testing for an evaluation of dietary adequacy in athletes is especially helpful for nutrients involved in oxygen transport (such as iron, vitamin B₁₂, folic acid) and vitamin D as well as electrolytes under certain conditions (for example, eating disorders and hyponatremia).

Finally, adequate fluid replacement, during and after exercise, can accurately be assessed using pre- and postexercise weight measures. To evaluate hydration

status before exercise and to monitor daily fluid balance can involve daily weight measurements, urine volume, urine color, and urine specific gravity.²²

To summarize, dietary adequacy can only be evaluated if an athlete's sport and associated energy demands are well understood and assessed. In addition, energy and nutrient intakes need to be estimated using as much accuracy and precision as possible without overburdening the athlete. Internet-based software can be integrated not only to assess a person's diet but also to evaluate energy expenditure. While the USDA Food Guide Pyramid software (www.MyTracker.gov) provides a simple approach to evaluating an individual's diet relative to activity level, sports-performance software is currently available that can be used to evaluate energy balance and nutrient adequacy in the context of the daily training plan (for an example, see www.trainingpeaks.com). These software programs are used interdisciplinarily among coaches and sports dietitians and provide a platform for online interaction with the athlete regarding training and meal planning. Although not yet sophisticated enough, these programs may offer innovative approaches to evaluating dietary adequacy in athletes in the future.

Before examining an athlete's diet, however, the sports dietitian must understand current recommendations for energy, nutrients, and fluids in sports and exercise. [Section 2.6](#) provides a summary of current guidelines.

2.6 DIETARY RECOMMENDATIONS FOR SPORT AND EXERCISE

2.6.1 ENERGY INTAKE

Energy intake should support the variability of the athlete's annual training and competition plan to bring dedicated months and years of training to fruition with expected performance outcomes. Estimating energy requirements is difficult to accomplish in the field, especially in sports that are less well studied. Examining the literature on DLW, conducted in athletes in free-living conditions, shows that energy expenditure can be quite high (see [Table 2.1](#)). What these studies also show is that there is a mismatch between energy expenditure and EI in the absence of weight loss, indicating the inherent bias of dietary assessment methods to underreporting.^{5,7,41}

Unfortunately, only few sports have been studied using DLW; thus, there are many sports for which it is difficult to estimate total daily energy expenditure (TDEE) mainly due to the difficulty estimating EEE. The aforementioned approaches (see [Section 2.5](#)) prove useful, but the specific method used depends on the athlete and the sport. If a quick reference is needed to establish a baseline for an athlete, the paper by Economos⁴² can also be useful. Energy requirements to support daily training for female and male athletes exercising approximately 90 minutes per day or less was suggested at 45 kcal·kg⁻¹·d⁻¹ and 50 kcal·kg⁻¹·d⁻¹, respectively. This may be a helpful target for sports dietitians working with athletes. However, these values do not suffice to quantify more reliably TDEE in athletes, and several of the above studies ([Table 2.1](#)) have shown that energy turnover in certain sports can be high and easily exceed 50 kcal·kg⁻¹·d⁻¹.

Using EI data from dietary records in combination with stable body weight has been suggested as a tool to determining energy needs of athletes. Typical EI data

assessed by 3- to 7-day dietary records that were reviewed by Burke⁴³ showed that female distance runners reported eating 2195 kcal·d⁻¹ (43 kcal·kg⁻¹·d⁻¹), while female strength and power athletes in track and field documented an EI of 2510 kcal·d⁻¹ (39 kcal·kg⁻¹·d⁻¹). Male endurance athletes reported a mean daily EI of 3320 kcal·d⁻¹ (56 kcal·kg⁻¹·d⁻¹) and strength and power athletes in track and field sports reported 3893 kcal·d⁻¹ (42 kcal·kg⁻¹·d⁻¹). That these data can accurately reflect energy requirement of athletes should not be assumed, however. Several factors need to be considered when using dietary intake data to estimate TDEE in athletes. As mentioned in [Section 2.2](#), underreporting of dietary intake occurs in individuals and groups.⁴⁴ In fact, in the DLW study by Edwards et al.²⁸ an energy discrepancy was found ranging from 4 to 58% in female runners. Especially in sports that emphasize leanness and thinness, it should be expected that EI is underreported. Further, ingesting significantly less energy than what the athlete's training load (that is, intensity × volume) would predict may also result in energy efficiency, where athletes maintain stable body weights despite eating significantly less than their energy expended. Thus, using EI data from dietary records is a less than optimal method for the prediction of energy requirement and to establish energy recommendations for athletes training and competing throughout the year. To increase accuracy and precision in determining an athlete's energy demand, professionals therefore should use the assessment techniques discussed previously and elsewhere in this volume, especially focusing on EEE, as it likely represents the largest variability of TDEE in athletes.

Recommendations to meet high-energy demands in sports depend on many factors, namely the sport itself and the changes in volume and intensity throughout training and competition. Simple strategies to meet high-energy demands during intense training are summarized in [Table 2.3](#).

Other factors that should be considered when making recommendations to meet high-energy demands include environmental conditions (for example, heat, cold, and altitude), endocrine issues (such as thyroid function and menstrual regularity), restrictive and disordered eating to achieve and maintain or reduce body weight, and weight and body composition goals.

2.6.2 CARBOHYDRATES

Carbohydrates provide energy for performance and recovery and exhibit a protein-sparing effect.⁴⁵ Athletes undergoing prolonged, intense, repetitive training require a high CHO intake of 7–10 g·kg⁻¹·d⁻¹ and up to 12 g·kg⁻¹·d⁻¹ if subjected to high training loads. When training at submaximal intensity and for shorter periods of time, athletes should target a CHO intake of 5–7 g·kg⁻¹·d⁻¹.^{15,19,46} Most elite athletes, training 5–6 hours per day, need a high CHO intake of between 7 and 12 g·kg⁻¹·d⁻¹, which in absolute terms ranges from 420 to 720 g of CHO per day for a female athlete weighing 60 kg. Particularly for female endurance athletes, such high CHO intakes are quite difficult to achieve. This was shown by Burke et al.,⁴⁷ who reported that female endurance athletes ingest on average 5.5 g·kg⁻¹·d⁻¹, whereas their male counterparts report a mean CHO intake of 7.5 g·kg⁻¹·d⁻¹. That CHO intakes around 5 g·kg⁻¹·d⁻¹ can sustain intense training and ensure adequate recovery of muscle glycogen stores is currently unclear but it appears highly unlikely. In fact, athletes engaging in intense

TABLE 2.3
Strategies to Increase Energy Intake in Athletes to Meet High-Energy Demands

Area of Focus	Strategies
Frequency of eating	Athletes should be advised to eat three to four meals and two to three snacks per day. Snacks are predominantly consumed before, during, and after exercise, between meals, and after dinner.
Meal size	Athletes should add calories to meals, which can be accomplished by adding fruit juice, sport drink, or milk as energy-containing fluids, by including an appetizer or dessert, or adding calories from foods that add flavors such as olive oil, nuts, seeds, cheese, bean spreads, or avocados.
Fueling before, during, and after exercise	Athletes should become skilled in selecting foods and fluids before, during, and after exercise to (1) optimize performance during and maximize recovery after exercise and (2) to meet the energy demands of intense training/competition and environmental extremes. Most athletes consume a significant amount of calories during the actual training or competition period.
Travel nutrition	Athletes should also learn how to prepare for travel, bringing foods and fluids to accommodate energy and dietary needs on the road or in the air, and to prepare a travel pack with foods to ease the transition to unfamiliar foods and jet lag and to get ready for competition.
Appetite and GI issues	Athletes should be sensitive to changes in appetite and gastrointestinal issues while training and competing. Decreased appetite may coincide with intense training, travel, and race preparation. Structured eating and meal plans can assist athletes to meet their energy needs during heavy exertion and in preparation for competition.
Illness and injury	Athletes should use strategies to meet energy demands when ill or injured (increase or decrease EI). Particularly when hospitalized with a traumatic injury (such as a fractured femur), athletes should refrain from restricting EI, because this likely interferes with early repair and rehabilitation.

training seem to sustain daily running performance better with a higher CHO intake at 8.5 g·kg⁻¹·d⁻¹ compared with a lower CHO intake of 5.4 g·kg⁻¹·d⁻¹.³⁸ Carbohydrates are also important to reduce mental fatigue, and data show that an athlete’s mood state is maintained more consistently on a higher CHO diet during intensified training.³⁸ Because endurance athletes are probably at greatest risk for inadequate CHO intake, it is important to educate them regarding the importance of increasing CHO intake during intense training periods. That cross-country skiers can manage this well was shown by the study of Sjödín et al.³¹ During an intense training period on snow, female and male Nordic skiers reported eating a high CHO diet consisting of 12 g·kg⁻¹·d⁻¹. This study also measured TDEE using DLW (see Table 2.2) and found that athletes were able to maintain energy balance and weight despite a high energy turnover of 80 kcal·kg⁻¹·d⁻¹. This study is a great example showing that female and male athletes should dare to eat more when EEE is high. Inadequate CHO intakes during intense training can lead to insufficient muscle glycogen restoration and delayed recovery, early fatigue during training and competition, and increased risk for illness and injury.^{19,46}

High CHO diets have also been shown to increase intermittent exercise performance,^{48,49} and several field studies in nonendurance sports have documented significant glycogen breakdown during training.^{50–52} However, these athletes do not necessarily eat a higher CHO diet. Data show that male and female strength and power athletes report consuming on average 5.5 and 4.7 g·kg⁻¹·d⁻¹ of CHO, respectively, and winter sport athletes report a CHO intake barely meeting 7 g·kg⁻¹·d⁻¹ during intense training.⁵³

Meeting CHO needs is often accomplished by the additional calories consumed before, during, and after exercise. Especially the period during exercise offers a great opportunity to maintain CHO adequacy to support daily training and recovery and to fuel performance. Brouns et al.^{54,55} were the first to document the calories consumed by Tour de France athletes while exercising. Today, CHO consumption during exercise should be optimized using foods and fluids that are easily absorbed and readily available to fuel working muscle during variable types of activities. Gastric emptying⁵⁶ and absorption across the intestinal wall⁵⁷ are probably the most important limiting factors of the muscle's capacity to oxidize CHO during exercise. Carbohydrate ingestion at 30–60 g per hour has been shown to increase endurance performance.⁵⁸ These amounts are typically available in sport drinks (6–8%). Although mixtures of glucose, sucrose, and fructose are better absorbed in combination due to specific transporters,⁵⁷ at low ingestion rates (< 60 g per hour), the type of sugar does not appear to matter.⁵⁹ At higher ingestion rates, a combination of sugars is probably more effective.⁵⁷ Carbohydrate ingestion during exercise is especially important if CHO consumption pre-exercise was insufficient and the athlete is hungry or thirsty. Carbohydrate intake during exercise should begin early and continue throughout the session for optimal benefits.^{19,46}

Carbohydrate intake 3–4 hours before exercise enhances liver and muscle glycogen synthesis and improves subsequent performance.^{17,19,46} Thus, much emphasis should be put on the pretraining and pre-event meal, containing between 200 g and 300 g of CHO, when teaching athletes about proper fueling for training and competition. Pretraining/event meals must be rich in CHO, low in fat and fiber, moderate in protein, adequate in fluid, and familiar. Although performance effects are unclear, many athletes consume a small snack 30–60 minutes before exercise. Due to the inverse relationship between the timing of CHO intake relative to the beginning of exercise and the quantity ingested, athletes should be advised to keep their snacks small when eating shortly before exercise. Finally, endurance athletes may also profit from high CHO intakes in the days prior to competition through CHO loading. In general, CHO loading increases muscle glycogen concentration and/or improves performance.^{19,46}

Carbohydrate after exercise is necessary to replenish muscle and liver glycogen in a timely fashion. Factors that are known to optimize glycogen resynthesis include the timing⁶⁰ of CHO intake, amount⁶¹ of CHO intake, and the type⁶² of CHO intake. It is recommended to ingest CHO within close proximity to exercise and at regular intervals to maximize glycogen resynthesis rate and optimize recovery, especially if another workout is planned within 8 hours.¹⁵ According to Rodriguez et al.,^{19,46} 1–1.5 g·kg⁻¹ of CHO within the first 2 hours and at 2-hour intervals for up to 6 hours are recommended (although less CHO at more frequent intervals is also effective).⁶¹ Sports dietitians should adapt recovery strategies to individual athletes to fit their postexercise recovery infrastructure.

2.6.3 PROTEIN

The RDA of protein is set at 0.8 g·kg⁻¹·d⁻¹ and the AMDR ranges from 10 to 35% of EI.¹⁴ Most of the higher needs for athletes have been established using nitrogen balance studies.⁶³ The protein recommendations for endurance athletes are higher than the RDA because protein oxidation is accelerated during prolonged and high-intensity exercise.⁶⁴ Protein is also needed for strength and to power athletes in excess of the RDA because additional amino acids are needed to support muscle growth and repair.⁶⁵ Protein recommendations for athletes are shown in Table 2.4.

Even though both endurance and strength training reduce protein requirements due to enhanced efficiency of protein utilization in athletes, protein recommendations remain unchanged. Important, however, is that individuals starting an exercise program would probably benefit from extra protein as shown in Table 2.3.^{19,46}

Protein intake within short proximity of exercise has the ability to enhance recovery and repair of muscle tissue. Timing of intake, type of protein, and the addition of other macronutrients such as CHO have all been investigated.²¹ Data show that the intake of 10–20 g of intact protein high in essential amino acids (EAA) or as little as 6 g EAA along with CHO postexercise can positively influence net protein balance after endurance⁶⁶ and resistance exercise.^{66–68} While most protein shakes exceed what can be incorporated into muscle tissue, individuals are advised to prefer food or manufactured recovery products with protein and CHO combinations over protein powders to support the recovery process postexercise. A thorough dietary assessment should provide the basis for recommendations given to athletes regarding daily protein requirements.

2.6.4 FAT

There are currently no specific fat recommendations or an RDA set for the general public or the active individual. The AMDR for fat is 20–35% of EI.¹⁴ Athletes should aim to distribute their fat calories among saturated and unsaturated fat based on the Dietary Guidelines for Americans and consume essential fatty acids as recommended by the DRIs.¹⁴ Fat requirements increase in athletes with increased levels of

TABLE 2.4
Protein Recommendations for Endurance and Strength Athletes

Sport/Activity	Recommended Amounts
Endurance	1.2–1.4 g·kg ⁻¹ ·d ⁻¹
Strength and power	1.2–1.7 g·kg ⁻¹ ·d ⁻¹

Sources: Rodriguez, N.R., DiMarco, N.M., and Langley, S., Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine, Nutrition and athletic performance, *J. Am. Diet Assoc.* 109(3), 509–27, 2009; Rodriguez, N.R., Di Marco, N.M., and Langley, S., American College of Sports Medicine Position Stand, Nutrition and athletic performance, *Med. Sci. Sports Exerc.* 41(3), 709–31, 2009.

training volume and intensity. Considering that some endurance athletes expend over 5000 kcal·d⁻¹, increasing fat intake proportionally is often necessary to meet such high-energy demands. A higher fat intake may also be needed to replenish intramuscular triglycerides (IMTGs) after prolonged exercise.⁶⁹

2.6.5 MICRONUTRIENTS

Although vitamins and minerals are not a source of energy, they play a vital role in energy metabolism and overall health. RDAs for vitamins and minerals are defined to prevent nutrient deficiencies and are not determined based on physical activity levels.⁹⁻¹² In general, athletes have adequate intakes of vitamins and minerals as long as EI is appropriate. However, B vitamins, calcium, vitamin D, iron, some antioxidants (for example, vitamins C and E, beta carotene, and selenium), zinc, and magnesium can be of concern. Athletes who present with compromised micronutrient status may be those who restrict energy intake for weight control or performance; eliminate one or more food groups due to dietary regimens, restrictions, or fear of calories; or consume unbalanced diets characterized by low micronutrient density.^{19,46}

Adequate intakes of B vitamins are essential to support the energy demand of daily training, recovery and repair of tissues. Data show that restrictions of thiamin, riboflavin, and vitamin B₆ with resulting marginal deficiencies have the potential to decrease aerobic capacity in trained male cyclists.⁷⁰ Although no single B vitamin was responsible for these results, this study at least illustrates that B vitamins are necessary for optimal performance. Thiamin, riboflavin, niacin, and vitamin B₆, among others, are directly involved in energy metabolism, while folic acid and vitamin B₁₂ function to support red blood cell synthesis, tissue repair, and maintenance of the nervous system. Vegetarian and vegan athletes are at greatest risk for low intakes of vitamin B₁₂ and riboflavin, and female athletes are also at risk for low intakes of folic acid and Vitamin B₆, in addition to the aforementioned B vitamins.^{71,72}

Adequate calcium intake is important in building optimal bone strength, especially at young age.⁷³ Insufficient calcium and vitamin D intake increases the risk of low bone mass and stress fractures in athletes.³⁵ Female athletes are at greatest risk for low bone mass, especially if EA is low and they present with menstrual dysfunction as part of the female athlete triad.³⁵ Most athletes need to increase their calcium intake to meet the RDAs, and this can be achieved by incorporating dairy products, calcium-fortified fruit juice, and soy products fortified with calcium sulfate. If athletes are also diagnosed with low vitamin D status, a calcium and vitamin D supplement may be necessary. Vitamin D deficiency has received a great deal of attention in the general public in the last few years and data show that athletes are also at risk.^{74,75} Athletes living in northern latitudes, who are minimally exposed to sun year-round and who train predominantly indoors are at greatest risk for vitamin D insufficiency and deficiency.^{19,46} In addition, individuals with pigmented skin (for example, African Americans) are at greater risk for compromised vitamin D status.⁷⁶ Vitamin D is also involved in skeletal muscle metabolism and immune and nervous system function,⁷⁴ and thus vitamin D deficiency in an athlete may affect any or all of these systems, although only limited data are currently available.

One of the most common micronutrient deficiencies in athletes is iron deficiency. Athletes most likely to suffer from low iron status include rapidly growing athletes, adolescent female athletes, female athletes with heavy menstrual losses, athletes on energy-restricted or meat-restricted diets, distance runners who may have increased gastrointestinal bleeding, and those training in environmentally challenging conditions (such as heat, altitude).^{19,46} While iron deficiency anemia compromises athletic performance,⁷⁷ it is unclear whether earlier stages of low iron status affects performance. Most athletes do not present with iron deficiency anemia but suffer from iron depletion and iron deficiency without anemia. It has been shown that supplementation in previously iron-deficient females improves iron status and has the potential to enhance aerobic performance parameters such as VO_2max .⁷⁸ Thus, early screening during preparticipation physicals allows for quick interventions using dietary modification and possibly low-dose supplementation.

Antioxidant nutrients, including vitamins and minerals such as vitamin C, vitamin E, beta carotene, and selenium, protect cell membranes from oxidative damage.⁷⁹ Chronic exercise is thought to increase oxidative stress because of the 10–15-fold increase in oxygen consumption during exercise. Thus, athletes training and competing year-round may have an increased need for antioxidant nutrients. There is, however, little evidence that antioxidant supplementation enhances performance. Athletes should be cautious with high doses of these micronutrients because they may result in counterproductive effects such as pro-oxidation and hampered training adaptation.⁸⁰ Athletes at greatest risk for low antioxidant intakes are those restricting energy and fat and lacking adequate fruit, vegetable, and whole grain consumption. Finally, the best antioxidant protection is probably offered by regular exercise⁸¹; data show that even during intermittent altitude training, athletes are not necessarily at greater risk for oxidative damage,⁸² and antioxidant supplementation does not significantly alter oxidative stress markers associated with increased energy expenditure at altitude.⁸³

A few other micronutrients that are typically low in athletes' diets include zinc and magnesium, especially in female athletes; vegetarians; and those on energy-restricted diets. Zinc's functions extend from energy metabolism to growth, muscle tissue repair, and immune function. Low zinc status is particularly prevalent in those on energy-restricted diets or vegetarian and vegan diets due to limited zinc content in plant foods. These diets are also high in fiber, potentially further decreasing zinc absorption.⁸⁴ Plasma zinc levels poorly reflect zinc deficiency⁸⁵; thus dietary assessments and certain physical symptoms such as loss of appetite, fatigue, and reduced performance may be better indices to identify mild zinc deficiencies in athletes. It is important to note that using zinc supplements can interfere with iron and copper absorption⁷¹; hence athletes are best served increasing their consumption of dietary sources of zinc or cover their zinc needs through a multivitamin/mineral supplement not exceeding the RDA for zinc.

Magnesium plays a multitude of functions in energy metabolism and regulation of membrane stability, cardiovascular, neuromuscular, immune, and hormonal functions⁷¹; therefore magnesium is a very important micronutrient for athletes. Low magnesium intakes are prevalent in athletes restricting energy intake most likely in endurance, weight-class, and aesthetic sports. Magnesium deficiency can lead to

impaired endurance performance.⁷¹ Exercise-induced muscle cramps in athletes are often perceived to be due to poor magnesium status. However, athletes should first assess hydration status and electrolyte balance before considering magnesium as a potential factor associated with cramping.

Athletes quickly opt for vitamin and mineral supplements before covering their micronutrient needs through foods such as fruits, vegetables, and whole grains. It has to be emphasized with this population that (1) supplementation in the absence of deficiencies does not increase performance,^{19,46} (2) too much of certain vitamins and minerals can have counterproductive effects,⁸⁰ and (3) dietary supplements may be contaminated⁸⁶ or mislabeled,⁸⁷ increasing the risk for adverse health effects or a positive drug test. Thus a thorough nutritional assessment, including blood parameters, is still the best foundation on which recommendations should be based for the increased nutrient intake from food and supplements to meet micronutrient needs in sport.

2.6.6 FLUID

Several documents have been published related to hydration guidelines by organizations such as the American College of Sports Medicine (ACSM), among others. The following list provides a few highlights from ACSM's evidence-based Position Statement on Hydration and Fluid Replacement²²; available at www.acsm.org:

1. Exercise can elicit high sweat rates and result in substantial water and electrolyte loss.
2. Sweat rates vary considerably between individuals and different sports.
3. Dehydration (> 2% body weight) can degrade aerobic exercise performance, especially in the heat.
4. Dehydration is a risk factor for exertional heat illness.
5. Body weight changes can reflect sweat loss during exercise and can be used to estimate individual fluid replacement needs for specific exercise and environmental conditions.
6. Fluid consumption that exceeds sweat rate is the primary risk factor for exercise-associated hyponatremia.

Sports dietitians working with athletes must individualize hydration guidelines, especially targeting fluid replacement during and after exercise based on pre- and postexercise weight measurements. In preparation for exercise, ACSM recommends ingestion of 5–7 mL·kg⁻¹ of fluid 4 hours before exercise and if urine is dark to drink an additional 3–5 mL·kg⁻¹ 2 hours before. During exercise, the athlete is advised to replace enough fluid to avoid excessive dehydration (> 2% in hot weather) and avoid drinking in excess of sweat rate. To optimize rehydration post exercise, fluid intake must exceed sweat loss. In fact, data by Shirreffs et al.⁸⁸ have shown that up to 150% of weight lost due to sweat loss during exercise should be replaced post exercise. Sport drinks pack CHO and electrolytes that, in combination, benefit fluid absorption, and sodium helps fluid retention.^{22,88} Thus sport drinks offer a great avenue to adequately hydrate and fuel before, during, and after exercise, especially when exercise occurs at environmental extremes.

2.7 FUTURE RESEARCH AND DIRECTIONS

Evaluating dietary adequacy in athletes is most likely limited by the assessment methods currently available to sports dietitians working in the field. Thus future research should aim at developing EI and expenditure methods that are more reliable and valid, efficient, and less burdensome for the athlete. Although sports dietitians have plenty of sports nutrition recommendations available from position papers and materials alike, as discussed in this chapter, there are only few benchmark values that help the professional evaluate quantity, quality, and timing of food intake in athletes under various conditions. Future research should include more studies in athletes focusing on macro- and micronutrient needs during specific phases of the annual training and competition plan. For example, CHO needs for high-intensity training phases should be targeted. Protein requirements should be identified for athletes needing to maintain lean tissue mass, strength, and power while on energy-reducing diets to lose body weight and fat. In addition, optimal fat intake should be identified for athletes, especially in endurance sports and in athletes on energy-restricted diets. And finally, micronutrient needs for athletes who expend high amounts of energy but restricting EI due to weight management plans should also be researched.

2.8 CONCLUSIONS

Good nutrition, appropriately timed, can improve the health and performance of an athlete or active individual. Determining an athlete's energy needs can be a challenging but necessary step to ensure that adequate energy and nutrients are consumed to maximize training potential and performance. The role of the sports dietitian is to keep abreast with current national and international dietary guidelines and reference intakes for the general public along with more specific recommendations aimed at physically active and athletic individuals in order to accurately evaluate the dietary adequacy of athletes.

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Section II

Anthropometric Assessment of Athletes

3 Physique Assessment of Athletes

Concepts, Methods, and Applications

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3.1 INTRODUCTION

A relationship between competitive success and physique traits has been identified in an array of sports, including football codes,¹ aesthetically judged sports,² swimming,³ track and field events,⁴ and skiing,⁵ as well as lightweight⁶ and heavyweight rowing.⁷ The specific physique traits associated with competitive success vary with the sport. For athletes participating in aesthetically judged sports, maintenance of low body-fat levels is associated with positive outcomes.^{2,8,9} A similar relationship

exists in sports where frontal surface area, power-to-weight ratio, and/or thermoregulation are important.¹⁰ However, in sports demanding high force production, muscle mass may be more closely associated with performance outcomes.^{1,3} Likewise, in sports such as rowing, other physique traits like a shorter sitting height (relative to stature) and longer limb lengths are related to competitive success,¹¹ with such information used successfully in talent identification.¹² Because of these relationships, it has become common practice to monitor physique traits of athletes in response to growth, training, and dietary interventions.

Despite the association between physique traits and competitive success, the assessment of body composition among athletes, especially female athletes and dancers, has been questioned due to the possibility of assessments promoting anxiety and disordered eating.¹³ This comes despite recognition that evidence supporting a causal relationship between body-composition assessments and disordered eating has yet to be established. Furthermore, when undertaken in conjunction with a suitably designed education program, current evidence indicates physique assessments can be undertaken without promoting adverse affective consequences.¹⁴ The concurrent education of athletes on the rationale for assessments makes good sense and should be actively promoted.

An array of techniques are available for the measurement of body composition, including anthropometric, radiographic (computed tomography [CT], magnetic resonance imaging [MRI], dual energy x-ray absorptiometry [DXA]), metabolic (creatinine, 3-methylhistidine), nuclear (total body potassium, total body nitrogen), and bioelectrical impedance analysis (BIA) techniques. When selecting the most appropriate technique, a range of factors should be considered, including technical issues such as the safety, validity, precision, and accuracy of measurement. Practical issues must also be considered, such as availability, financial implications, portability, invasiveness, time effectiveness, and technical expertise necessary to conduct the procedures. Consideration must also be given to the ability of body-composition assessment methodologies to accommodate the unique physique traits characteristic of some athletes, including particularly tall, broad, and muscular individuals or those with extremely low body-fat levels.

This chapter reviews the most common techniques used to assess the physique traits of athletes, including DXA, air displacement plethysmography (ADP), BIA, and surface anthropometry. A review of newer techniques with potential application to athletic populations either directly or via research investigations is also made, as well as discussion on the factors that should be considered when attempting to minimize measurement error.

3.2 HYDRODENSITOMETRY

Hydrodensitometry, or underwater weighing (UWW), has long been considered the gold-standard method for assessing body composition. The technique is based on Archimedes' principle that body mass in air compared to body mass when totally submerged in water is directly related to the density of the water displaced.¹⁵ Technically, it simply demands the measurement of an individual's body mass both in and out of the water with a correction for residual lung volume (RV). The volume of water displaced can be calculated from the known density of water at any given

temperature (1 g of water = 1 cm³ at 39.2°F [4°C]).¹⁵ As this does not account for the RV of air left in respiratory passages and lungs, and gas in the gastrointestinal tract, a correction for gas volume is necessary. This can either be estimated based on the subject’s age, height, and mass or measured via an array of dilution techniques, including oxygen¹⁶ and helium dilution.¹⁷ The underwater weighing procedure is usually repeated eight to twelve times until three trials are obtained with results within 100 g of each other.¹⁸ The average of the three trials is used to calculate body density.¹⁹ A more recent study has reported minimal error from four trials with automated data acquisition and in-tank residual volume measurement.²⁰

Failure to account for the RV will underestimate whole-body density, as this air contributes to the buoyancy effect. Gas in the gastrointestinal tract cannot be estimated with accuracy and is often disregarded. However, the volume of gas can be significant after ingestion of certain foods and medical conditions.²¹ The results obtained from mass in air, mass in water, and RV can be used to calculate body density using the following equation²¹:

$$BD = \frac{Wa}{\left(\frac{Wa - Ww}{Dw}\right)} - V$$

where *BD* = body density, *Wa* = weight in air, *Ww* = weight in water, *Dw* = known density of water for the temperature at which *Ww* was obtained, and *V* = residual lung volume.

Body density derived from hydrodensitometry can be used to estimate the relative fat content of the human body and is based on the principle that water, fat, protein, and mineral have different but constant densities.²¹ The density of each body component was derived from calculated volumes developed by Brozek and colleagues²² that were based on autopsy data of just four human cadavers (Table 3.1). Although cadaver studies are the only true direct method of measuring body composition, they also have limitations due to the technical difficulty with dissection,²¹ the unknown impact of cause of death on body composition,²³ and the application of results from such small samples of geriatrics to a much younger, athletic population.

TABLE 3.1
Density of Four Human Body Components

Body Component	Density (at 37°C)	Comment
Fat	0.9007 g.cm ⁻³	Constant via body site May vary between individuals Includes all substances with same density as triglycerides (TG)
Water	0.993 g.cm ⁻³	May contain solutes of protein and inorganic salts
Protein	1.340 g.cm ⁻³	Value for fully hydrated protein <i>in vitro</i>
Mineral	3.000 g.cm ⁻³	Value <i>in vitro</i>

Sources: Adapted from Brozek, J., Grande, F., Anderson, J.T., and Keys, A., *Ann. N.Y. Acad. Sci.* 110, 113–40, 1963; and Siri, W.E., *Nutrition* 9(5), 480–91, 1993 (originally published in 1961).

The relationship between body density and fat shown by Behnke²⁵ in 1942 led to the derivation of equations for the calculation of percent body fat as follows:

$$\% \text{ body fat} = (495/\text{body density}) - 450^{22}$$

$$\% \text{ body fat} = (457/\text{body density}) - 414.2^{24}$$

These equations have been used extensively to calculate body fatness in both laboratory studies and in the field.²⁶ They yield similar results for body densities ranging from 1.030 to 1.090 g.mL⁻¹.²⁷ However, there are a number of assumptions associated with the equations that have been largely ignored with their application. Formulas for estimating fat from density is based on the premise that all adult humans are identical in composition except for differences in proportion of adipose tissue^{21,22} (that is, a two-compartment model of fat mass [FM] and fat-free mass [FFM]), and on assumptions about the consistency in the chemical composition of FFM. For example, it is assumed that there is a constant ratio of water, protein, and mineral of 73.7%, 19.4%, and 6.8%, respectively.^{21,24} There is also an assumption that changes in body mass do not result in any change to the consistency of the FFM. However, adipose tissue consists of lipid, water, and protein, not fat alone, and changes in body mass result in changes to all components.²⁴

The fat-mass density assumption is based on the average of 0.9007 ± 0.00068 g.cm⁻³ for twenty ethyl ether extracted fat samples from the intra-abdominal and subcutaneous tissue of just five subjects.²⁸ However, it has been suggested that the small coefficient of variation validates the hydrodensitometric fat-mass density assumption of 0.9007 g.cm⁻³.²⁹ Similarly the hydrodensitometric fat-free-mass density assumption of 1.100 g.cm⁻³ is based on analysis of just three male cadavers aged 25, 35, and 46 years.²² Biological variation in FFM hydration away from the assumed 73.72% is particularly troublesome given that water has the lowest density but comprises the largest percentage of any FFM compartment. When total body water is measured directly via dilution techniques, water comprises 70.4%–75.1% of the FFM.³⁰ Consequently, hypohydration and hyperhydration, respectively, increase and decrease FFM density with associated under- and overestimation of percentage body fat via hydrodensitometry. Despite this, the assumed FM and FFM densities are applied irrespective of age, gender, genetic profile, and training status of individuals.²⁹

Siri recognized that hydrodensitometry did not account for biological variability between individuals and subsequently assessed the errors associated with the technique.²⁴ He concluded that estimates of absolute fat had an error of 4% and recommended that measurements of different components of the FFM (protein, mineral, and water) should be treated independently.²⁴ There is now substantial evidence suggesting that the use of a multicompartment model is essential to quantify differences in FFM.³¹ Subsequent studies have shown that failure to use other techniques to determine total body water and bone mineral density can lead to an inaccurate estimation of body fatness in children,³² the elderly,³³ and ethnic populations.³⁴ This is due to variation in density of FFM with age, gender, ethnicity, level of fatness, and activity level.^{35–37}

While it is often assumed body density derived from hydrodensitometry is a very robust measure, consideration must still be given to subject presentation. Within an

athletic population, acute variation in hydration status may need to be considered, with hypohydration shown to reduce estimates of body fat percentage³⁸ and FFM.³⁹ While the ingestion of 1.2 to 2.4 L of water does not influence measures of residual volume or underwater weight, body mass out of the water is increased. Body density is subsequently increased, resulting in a lower estimate of body fat percentage,⁴⁰ although this effect may not be evident when smaller volumes of fluid (0.5 L) are ingested.³⁸ Although the ingestion of food and fluid throughout the day on hydrodensitometry results has not been investigated, it is reasonable to presume larger volumes could also influence hydrodensitometry derived estimates of body composition because of their influence on body mass. This is aligned with the original work of Siri, indicating that formulas would become invalid in the presence of abnormal hydration.^{21,24}

Hydrodensitometry has recently declined in its popularity as a method to determine body fatness due to the introduction of simpler, faster techniques (ADP, BIA, and DXA). There are obvious potential limitations associated with subject anxiety during measurement, so familiarization is always essential. Although the application of this method is primarily limited to a research setting, it remains an excellent measure of body density despite some of the assumptions outlined above associated with the conversion of body density into body composition.

3.3 TOTAL BODY WATER

Measurement of total body water (TBW) is typically reserved for use in research studies on body composition as it requires dosing with a precisely measured quantity of a tracer (such as deuterium, oxygen-18, or tritium), moderate to sophisticated laboratory equipment (mass or isotope ratio mass or infrared spectrometry, gas chromatography, nuclear magnetic resonance, or scintillation counting, for example), and significant technical expertise to accurately analyze and interpret the results.⁴¹ The dilution of the tracer in body water can be used to calculate the total volume of body water. The simplest and most commonly adopted approach for estimation of body composition via TBW uses a two-compartment model of FM, which is free of water, as opposed to FFM, which is estimated to contain approximately 73% water.⁴¹ The calculation of FFM using TBW assumes that there is a constant hydration of the FFM compartment, that is, that the ratio of solid to water is the same in all individuals. Remarkably, early animal work by Pace and Rathburn,⁴² who first recommended the 0.73 FFM hydration constant, has been replicated by a number of adult human cadaver studies.^{43,44} This assumption, although reasonable in healthy individuals, may be altered in athletes who are hypohydrated as a result of fluid loss during training/competition or in clinical populations as a result of abnormal fluid loss or retention due to disease.

Key assumptions of TBW measurement by isotope dilution:⁴¹

- The tracer is not distributed in other body compartments, only in body water.
- Equal distribution of the tracer occurs in all anatomical water compartments.
- The tracer reaches equilibrium at a rapid rate.
- The tracer and body water are not metabolized by the body during the period of tracer equilibrium.

Although none of these assumptions are perfect, they are reasonable, and limitations depend on the methodology of dosing, measurement, and population assessed. Equilibration of the tracer typically takes at least 3–4 h and correction is required for exchange with nonaqueous hydrogen or oxygen.⁴¹ Precision of the method depends on the analytical approach used and tracer dose but generally mass spectrometric methods have high precision and accuracy within the range of 1–2%.^{45,46}

There are a number of different approaches to assessment of TBW but the “plateau method” is one of the most frequently used for body-composition assessment,⁴¹ although a back extrapolation method is also available.⁴⁷ In the plateau method, subjects typically fast overnight (8–12 h) and refrain from exercise (potentially in athletes for the previous 24 h) to prevent excessive insensible water loss. A baseline biological sample of blood, plasma, saliva, urine, or breath water vapor is collected prior to collection of nude body weight (when isotope mass spectrometry is used, an additional baseline sample should also be taken 24 h prior to dosing to assess day-to-day isotope variation). After baseline samples are collected, an oral weighed dose of the isotope is then administered with care taken to rinse the dosing container with additional plain water (~50 mL) to ensure it is entirely consumed.⁴⁸ During the following equilibrium period, the subject should remain nil by mouth. A subsequent biological sample is typically taken at 3, 4, and 5 h post dose. If urine is being used, subjects should void and discard a sample before collection of duplicate urine specimens at the above prescribed times. Samples should be stored in airtight containers to prevent loss of isotope until analysis. Refrigeration or freezing at –20°C is recommended to minimize bacterial growth. Sample enrichment of the two post-dose samples should agree within two standard deviations.

Precise measurement requires attention to subject preparation, dosing, sample collection, and isotope analysis. Subjects must present euhydrated and with normal glycogen stores,⁴¹ which can be challenging for some athletes. Specific dietary guidance for athletic subjects to ensure euhydration and glycogen repletion from at least 12–24 h before dosing is therefore important. The final meal before dosing should be consumed 12–15 h prior to the dose to minimize water content in the intestine. Subjects should also avoid drinking several hours before dosing to avoid overhydration.⁴¹ The subjects should also be rested in an environment that prevents excessive sweating.⁴¹ Given these constraints, early morning dosing is most convenient and reduces the discomfort of fasting. Doses are prescribed relative to body mass of subjects and need to be precisely weighed and transported in a nonpermeable, airtight container to minimize evaporation.⁴⁸ A sample of the dose diluted with tap water (so that the enrichment approximates the concentration in the physiological samples) should also be measured in the same batch along with the diluting water together with the samples.⁴⁹

Deuterium was the first tracer used for the measurement of TBW. As it is stable, safe, and relatively inexpensive, deuterium remains the most common choice for assessment of body composition.⁴¹ Analysis using tritium was popular for a time due to the availability of scintillation counters⁵⁰ and ease of measurement, but the disadvantage is exposure to a small radiation dose, and for this reason it is now rarely used. Oxygen-18 is stable and safe but by far the most expensive isotope for dosing and can only be accurately measured by mass spectrometry, which further increases

the cost. Use of this method in athletes is reserved for research, typically to assist with the validation of other body-composition techniques that have practical field use application (such as bioelectrical impedance). Those interested in this methodology are referred to the following resources for an in-depth coverage.^{41,47–49}

3.4 DUAL ENERGY X-RAY ABSORPTIOMETRY

DXA was originally developed for the diagnosis of osteoporosis and remains the gold-standard tool for this assessment.⁵¹ However, DXA technology is also able to measure soft-tissue body composition, rapidly gaining popularity in recent years as one of the most widely used and accepted laboratory-based methods for body-composition analysis. DXA not only provides a measure of FM and FFM, it also provides information on regional body composition (arms, legs, trunk, differences between left and right side), making DXA technology unique among physique assessment tools and particularly appealing among athletes when undertaking targeted training programs or during periods of rehabilitation from injury. Furthermore, whole-body scans are rapid (~5 min), noninvasive, and associated with very low radiation doses (~0.5 μ Sv or approximately 1/500th of annual natural background radiation), making the technology safe for longitudinal monitoring of body composition. Because of its application in the assessment of bone mineral density, DXA technology is also becoming increasingly available.

There are three manufacturers of DXA technology: Hologic Inc. (Waltham, Massachusetts), Lunar Radiation Corp. (Madison, Wisconsin), and Norland Medical Systems (Fort Atkinson, Wisconsin); all models share in common an x-ray source, scanning table, detector, and computer interface with complex algorithm software for the conversion of raw data into estimates of body composition. The systems differ in analysis software and the geometry of scanning, using either fan, narrow fan, or pencil beam technology, which ultimately determines scanning time, radiation dose, and accuracy.⁵² Because of these differences, longitudinal monitoring of athletes should be undertaken on the same machine^{53–55} and using the same technician,⁵⁶ especially if regional body-composition changes are of interest.

DXA technology is based on the differential attenuation of transmitted photons at two energy levels by bone, fat, and lean tissue.⁵⁷ Attenuation of low-energy photons are then expressed as a ratio to attenuation observed for the high-energy photons, the outcome of which is specific to different molecular components, including fatty acids, protein, and bone. In theory, assessment of all three components would require measurement at three different photon energies. The DXA dual-energy system can thus only be used to estimate the fractional masses of two components in any one pixel. That is, in bone-containing pixels, bone mineral and soft tissue can be measured, while in non-bone-containing pixels, fat and bone mineral-free lean mass can be measured.⁵⁸ The proportion of fat and bone mineral-free lean in bone containing pixels is assumed to be the same as the adjacent non-bone-containing pixels,⁵⁸ with the software subsequently incorporating individual pixel data into whole-body output. This assumed ratio of fat to bone mineral-free lean in soft-tissue pixels is applied to upwards of one third of pixels in a whole-body scan and particularly evident in regions

of low bone-free pixels such as thorax, arm, or head, resulting in the identification of composition changes in these regions as being less reliable.^{59,60}

DXA technology has been validated against the modern day “gold standard” body-composition assessment tool, the four-compartment model, which accounts for variation in the water and mineral fractions and the density of the FFM. Although there is some data suggesting good agreement between DXA-derived measures of body composition and the four-compartment model in healthy, young males and females,⁶¹ others have indicated that DXA underestimates body fat,⁶² especially among leaner individuals.^{30,63} This has been attributed to variation in FFM hydration⁶² or differences in anterior–posterior tissue thickness.⁶³ However, among athletes where the primary focus is on monitoring change in body composition, DXA appears to offer sufficient sensitivity to identify small changes in body composition.^{64,65}

The precision of measurement for DXA in sedentary populations has been shown to be superior to hydrodensitometry and surface anthropometry,⁶⁶ with a coefficient of variation of less than 1.0 kg for FM, FFM, and total mass.^{57,67} Any variability of results achieved by DXA can be divided into two categories: technical error or biological error. In an effort to enhance precision of measurement, special consideration should be given to subject positioning,^{68,69} with subjects resting on the scanner area in a supine position with special care taken to ensure their entire body fits within the specified scanning area. Where possible, positioning of the arms and legs should be standardized, ensuring clear separation from the torso; foam blocks not recognized by the scanner can be particularly helpful. Clothing should be kept to a minimum,⁷⁰ with all metal objects removed. While small amounts of food and fluid do not appear to influence results,⁷¹ larger volumes influence measurement of lean body mass,⁷² and thus measurements should be undertaken in a fasted state wherever possible, preferably soon after waking in a euhydrated state.⁷³ Reliability of regional measurements is inferior to total body results.^{69,74}

Of particular relevance to athletic populations is the defined scanning area available for assessment, typically within the range of 60–65 cm × 193–198 cm, depending on the manufacturer.⁵² It is therefore difficult to perform whole-body DXA scans on particularly tall or broad and very muscular athletes, physique traits common to some sports such as rowing, basketball, volleyball, and rugby union. Thus, taller individuals are either excluded from investigation, scanned without their head or feet, or have their knees bent so as to fit within the scanned area,⁷⁵ or data is summed from two partial scans, the latter appearing to be the method of choice with the body divided at the neck resulting in the most accurate estimates of bone and soft tissue composition.⁷⁶ Until recently, very broad individuals were “mummy wrapped” in a sheet, bringing the arms forward, so as to fit within the scanning area. While this afforded a whole-body scan to be undertaken, the number of bone-containing pixels is significantly increased and limits the ability to assess body composition at particular regions of interest, such as the arms and torso. Newer DXA instruments, like the iDXA from GE Lunar (Madison, Wisconsin), not only have larger scanning areas (66 cm wide) but also come with software that allows an estimate of whole-body composition from a half-body scan,⁷⁷ a concept validated previously in obese individuals.⁷⁸

3.5 AIR DISPLACEMENT PLETHYSMOGRAPHY

While ADP has been used to measure human body composition for some time, a viable system known by the trade name BOD POD (Life Measurement, Concord, California), has only been available commercially since the mid-1990s with a pediatric version (PEA POD) now also available. This quick, comfortable, automated, non-invasive, and safe technique provides an estimate of percent fat from body density without being submerged under water and accommodates a range of subject types,⁷⁹ including very tall and muscular athletes.⁸⁰ Air displacement plethysmography utilizes basic gas laws to describe the inverse relationship between pressure and volume in two enclosed chambers, consequently allowing for the calculation of body density and body composition.

This two-compartment technique involves sitting quietly in an enclosed chamber while the volume of air displaced by the body is measured. Duplicate measures of body volume are recommended, with volumes averaged if they differ by ≤ 150 mL, and a third trial undertaken if they are > 150 mL, with the closest two body volumes averaged.⁷⁹ Thereafter, lung functional residual capacity is measured using pulmonary plethysmography or if this is not possible (for example, in the elderly, children, or those with pulmonary dysfunction), predicted based on age, gender, and height. If no two measurements meet the acceptance criteria, the entire test procedure (including recalibration) should be repeated.⁸⁰ Body density is then calculated by dividing the measured body mass by corrected body volume, with subsequent calculation of percent body fat using either the Siri²⁴ or Brozek²² equations. As such, the BOD POD is constrained by the same issues as hydrodensitometry when converting a measure of body density into body composition.

The measurement of functional residual capacity using pulmonary plethysmography is both reliable and valid.⁸¹ However, it can change in response to significant adjustments in body composition and as such should be measured wherever possible and never used interchangeably with predicted thoracic gas volumes.⁸² Furthermore, on an individual basis, body fat can deviate by as much as 3% depending on the use of measured versus predicted lung volume.⁸³

The BOD POD compares favorably as a substitute for underwater weighing when a measure of body density is desired,⁸⁴ although it has been observed to underestimate body fat slightly in males ($-1.2 \pm 3.1\%$) and overestimate body fat in females ($1.0 \pm 2.5\%$), independent of age, weight, or height.⁸⁵ This effect is evident among athletic populations as well, with BOD POD–derived estimates of body fat consistently lower than those obtained via hydrodensitometry, DXA, and a three-compartment model for male collegiate football athletes.⁸⁶ Among female athletes, the BOD POD overestimates body fat when compared against hydrodensitometry but either compares favorably⁸⁷ or underestimates body fat when contrasted with DXA.⁸⁸ These differences between techniques should not be a surprise and are likely a consequence of methodological error of both techniques, including the presumed gold standard or established techniques.

Although the ability to assess absolute character traits is an important attribute of a physique assessment technique, equally important is the ability to identify small but potentially important changes in body composition in response to diet, training,

or other interventions. Research using the artificial manipulation of body composition by the addition of 1–2 liters of oil, water, or a combination of both substances to the BOD POD chamber among normal-weight individuals suggests the technology has the ability to pick up changes in either component within the range of 2 kg.^{89,90} When the BOD POD has been used in conjunction with DXA to monitor body-composition changes in response to lifestyle interventions, BOD POD estimates of fat mass are typically lower, with concomitant higher estimates of FFM.^{64,91} However, agreement between techniques was high for identifying the changes in physique traits in response to lifestyle interventions.^{64,91,92}

As with other physique assessment tools, subject presentation can influence results, and thus suitable protocols must be implemented to avoid the impact of these on the reliability of data. Specifically, uncompressed facial and scalp hair underestimate body fat due to trapped isothermal air in body hair.⁹³ Similarly, loose-fitting clothing worn during assessment influences body-density measurements, underestimating body fat percentage by upwards of 9%.^{84,94} Consequently, subjects are advised to wear standardized tight fitting swimsuits consistently^{84,94,95} in conjunction with a swim cap and to remove excess facial hair.⁹³ Changes in body temperature and moisture content may also influence BOD POD data,⁹⁶ suggesting that assessments should be undertaken independent of exercise. This is further supported by the fact that acute dehydration (within the range often experienced by athletes) influences BOD POD results, underestimating both body fat percentage and FFM, although the effect is within the range of 1% body fat.³⁹

Minimizing the influence of these variables enhances the ability of the BOD POD to track small but potentially important changes in body composition. While the test–retest reliability of the BOD POD is excellent,⁹⁷ this does not provide insight into the biological variability evident between test measures. The between-day coefficient of variation for body fat using the BOD POD is within the range of 2.0–5.3%,⁹⁸ although large discrepancies (up to 12%) between trials have been reported in a small percentage of individuals,⁹⁷ for reasons still yet to be determined (but could include variation in breathing patterns or transient change in pressure within the test room). In practice, technicians are encouraged to undertake repeat measurements and if these two tests show a difference in percent body fat greater than 0.5%, then a third test may be appropriate.⁸³ Although reliability between individual BOD POD systems is very good,⁹⁹ athletes should be encouraged to be assessed using the same machine each time.

3.6 BIOELECTRICAL IMPEDANCE

BIA is a safe and noninvasive method to assess body composition that is based on the differing electrical conductivity of FM and FFM.^{100,101} FFM contains water and electrolytes and is a good electrical conductor, while anhydrous fat mass is not. The method involves measuring the resistance (R) to flow of a low level (800 μ A) 50 kilohertz (KHz) current.¹⁰¹ Resistance is proportional to the length (L) of the conductor (in this case, the human body) and inversely proportional to its cross-sectional area (A). A relationship then exists between the impedance quotient (L^2/R) and the volume of water (total body water), which contains electrolytes that conduct

the electrical current. In practice, height in centimeters is substituted for length. Therefore, a relationship exists between FFM (approximately 73% water) and height $(\text{cm})^2 / R$. FM is obtained from FFM by subtracting the value for FFM from total body mass.¹⁰¹ Two types of resistance exist in the human body. One is resistive (R) as described earlier, and the other is capacitive or reactance (X_c). “Impedance” is the term used to describe the two types of resistance. As the electrical properties of tissue vary depending on nutritional status and hydration, the relationship between the two can be used to diagnose various disease states.¹⁰¹

Although the relationship between FFM and impedance is readily accepted, there are several assumptions associated with its use. First, the human body is assumed to be a cylinder with a uniform L and A .¹⁰² However, the human body more closely resembles several cylinders. The body parts with the smallest FFM (the limbs) have the greatest influence on whole-body R . The trunk, which is a shorter, thicker segment, contains 50% of body weight, but contributes a minor amount to the overall R .¹⁰² The second assumption is that the conducting material in the cylinder is consistent throughout. However, this will vary depending on tissue structure, hydration status, and electrolyte concentration of the tissue.¹⁰²

Early regression equations to calculate FFM only used height and resistance without considering many of these assumptions. More recently, other parameters such as body mass, age, gender, and anthropometric measures have been included to improve accuracy, resulting in numerous population-specific equations.¹⁰¹ BIA appears to give a reasonably accurate assessment of body composition in healthy individuals provided a validated equation with appropriate age, gender, and ethnicity compatibility is utilized.

Due to the relevance of body water to conductivity of electrical current, there is substantial evidence that BIA is not valid for assessment of subjects with abnormal hydration.¹⁰³ Hydration status is an issue pertinent to athletic populations. A study by Saunders and colleagues¹⁰⁴ found that changes in hydration status in endurance-trained individuals caused large fluctuations in percent body fat, with a 1.7% decrease from euhydration to hypohydration and 3.2% increase from hypohydration to complete rehydration, and a further 2.2% increase with hyperhydration (3% above normal body weight). The ingestion of smaller volumes of fluid (591 mL) also results in 1% increase in estimates of percentage body fat, suggesting acute fluid (and most likely food) intake can also increase measurement error,¹⁰⁵ prompting recommendations that subjects should remain fasting for at least 8 h prior to assessment.¹⁰³ Given this, it would be prudent to undertake assessments in the morning prior to breakfast wherever possible, with subjects encouraged to present in a well-hydrated state; assessment could be done via the collection of a first-morning urine sample.

The measurement of difference in electrical properties of various body tissues is not a new concept. The original studies were conducted by Thomasset in the 1960s using two subcutaneous inserted needles.¹⁰⁶ The technique was subsequently refined in the 1970s to four surface electrodes and resulted in commercially available single-frequency analyzers.¹⁰¹ Since that time, there have been significant advances in BIA with options of single frequency, multifrequency, and segmental BIA.

Foot-to-foot BIA analyzers are the most readily available for public purchase.¹⁰⁷ These inexpensive body fat “scales” are popular among the general public, being promoted as a simple, portable method of measuring body fatness. However, there are a number of limitations with foot-to-foot devices as current is only circulated through the legs and lower part of the trunk with results extrapolated to the whole body.¹⁰⁷ If used within an athletic population, “athlete” mode should be used where available when monitoring athletes.¹⁰⁸

Single-frequency BIA (50 kHz) is most commonly used as a field technique to measure body composition. They usually consist of four electrodes placed on the wrist and hand, plus ankle and foot, although foot-to-foot, and hand-to-hand analyzers are also available. The two source electrodes are placed on the dorsal surface of the right hand and foot proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints respectively. The two voltage electrodes are placed on the midpoint between the distal prominences of the radius and ulna of the right wrist and between the medial and lateral malleoli of the right ankle.¹⁰² Although this technique is relatively simple, less than optimal accuracy has been observed when compared to other techniques such as DXA and hydrodensitometry. Possible sources of error have been attributed to arm positioning, skin temperature, interobserver variability, and electrode placement.^{103,109} It is apparent that standardized protocols for use (Table 3.2) are essential to ensure that measurement error is minimized.

Multifrequency BIA consists of measurement of impedance at various frequencies (0, 1, 5, 50, 100, 200, and 500 kHz). At a low single frequency, an electrical current will not fully penetrate the cell membrane, passing through extracellular water, whereas at high frequencies the current will penetrate the cell membrane.¹¹⁰ By measuring various components across a number of frequencies, a mathematical model can be derived that can be used to predict TBW and subsequently FFM instead of the use of standard regression equations. This may be appropriate for individuals where there is variation in standard hydration status or body composition.¹¹¹ The results of various studies comparing multifrequency BIA to single-frequency BIA and other methods such as DXA have reported mixed results.^{112,113} Among individuals with low body-fat levels, BIA tends to overestimate fat mass and percentage fat mass while underestimating FFM.¹¹⁴

Bioelectrical impedance has become increasingly popular as a tool for assessing the physique traits of athletes given its relative ease of use, portability, and cost effectiveness. To be confident in the use of this technology to track changes in physique traits, it must be compared against an accepted tool for tracking changes in body composition. While both single-frequency and multifrequency BIA show good absolute agreement with DXA during a period of weight loss, large individual variance can occur,¹¹⁵ reinforcing the need to implement assessment practices that limit measurement noise.

3.7 SURFACE ANTHROPOMETRY

For reasons of timeliness, practicality, and cost effectiveness, the routine monitoring of body composition among athletic populations is often undertaken using anthropometric traits such as body mass plus subcutaneous skinfold thicknesses and girths at

TABLE 3.2**Recommendations for Standard Methodology for Bioelectrical Impedance Analysis**

Instrument	Recommendation
Generator	Regular calibration Battery powered to avoid interfering with current
Analyzer	Ability to identify abnormal skin resistance Identifies type of signal measured (i.e., resistive or reactance)
Cables	Appropriate for subject height (up to 200 cm) Diameter meets manufacturers recommendations
Electrodes	Meets instrument requirements ($> 4 \text{ cm}^2$) Keep electrodes in sealed bag and protect from heat
Subject	Recommendation
Ethnicity	Note ethnicity and use appropriate equations
Height and body mass	Measure at time of assessment; self-reported measures not valid
Bladder voided	Void before measurement
Hydration status	Assess upon waking urine sample to determine hydration status
Physical activity	Abstain for 8 h before measurement
Timing	Note time of day and replicate for subsequent measurement Note phase of menstrual cycle in women
Skin condition	Ambient temperature, no skin lesions at site of electrodes Clean with alcohol
Electrode position	Note side of body and repeat on the same side
Limb position	Abduction of limbs, arms 30° from trunk and legs separated at 45°
Body position	Standardize time before measurements (usually 5–10 min)
Body shape	Note any abnormalities
Environment	Measure in a thermo-neutral environment. No contact with metal

Source: Adapted from Kyle, U.G., Bosaeus, I., De Lorenzo, A.D., Deurenberg, P., Elia, M., Manuel Gomez, J., Lilienthal Heitmann, B., Kent-Smith, L., Melchior, J.C., Pirlich, M., Scharfetter, H., Schols, A.M., and Pichard, C., *Clin. Nutr.* 23(6), 1430–53, 2004.

specific anatomical landmarks. Unlike other techniques requiring expensive, laboratory-based equipment, surface anthropometry only requires relatively inexpensive equipment that is easily portable. However, highly skilled technicians are required if reliable data are to be collected. Technicians need to be particularly meticulous with both accurate site location and measurement technique. Measurements just 1–2 cm away from a defined site can produce significant differences in results.^{116,117} Furthermore, if repeat measurements are to be taken over time, it is important that the same technician collect the data.¹¹⁷

The measurement of skinfolds, or a double layer of skin and subcutaneous tissue, as an index of whole-body fat would appear to be reasonable. However, what is really being measured is the thickness of a double fold of skin and compressed subcutaneous adipose tissue (SAT).¹¹⁸ To infer from this the mass or percentage of total body fat requires a number of assumptions to be made, including

- Constant compressibility of skinfolds across sites on the body
- The skin thickness at any one site is negligible or a constant fraction of a skinfold
- Fixed adipose tissue patterning across the body
- A constant fat fraction in adipose tissue
- Fixed proportion of internal to external fat

When assessed via cadaver analysis, few of these assumptions hold true.¹¹⁹ For example, skinfold compressibility is not constant between sites and as a consequence, similar thicknesses of adipose tissue may yield different caliper values due to different degrees of tissue compressibility.¹²⁰ Furthermore, the patterning of adipose tissue varies markedly between individuals,¹²¹ and as such, multiple skinfold sites should be used, including both upper- and lower-body landmarks.¹²² Similarly, while it is estimated that subcutaneous fat comprises one third of total body fat, this can range from 20% to 70% depending on gender, degree of fatness, and age.²⁷ Despite an obvious violation of these assumptions, a strong relationship does exist between subcutaneous adiposity and whole-body adiposity, and between direct skinfold thickness measures and whole-body adiposity.¹¹⁹

Estimates of body density, FM, and FFM can then be derived from raw skinfold data using one of many available regression equations. Altogether, more than 100 equations to predict body fat from skinfolds have been produced.^{27,118,123,124} However, these equations are typically based on a single-measurement, between-subject, cross-sectional comparison of anthropometric parameters and laboratory-based techniques such as hydrodensitometry,¹²⁵ increasing the assumptions made. Because these equations are population specific, only equations derived from individuals with similarities in age, gender, body composition, and activity levels should be considered for use. Furthermore, compatibility in technical aspects of data collection, including anatomical landmarking and anthropometric equipment is also essential. Consequently, among athletes, skinfold equations derived from athletic populations such as that of Withers et al.¹²⁶ are more likely to offer a more accurate estimate of body composition.¹²⁷ However, the ability of these equations to track changes in physique traits in response to training and/or dietary interventions has not been widely assessed.^{125,128} Preliminary data suggests popular skinfold-based models, including those derived from athletes, lack the sensitivity to track small but potentially important changes in body composition.^{129,130} As such, it seems unreasonable to introduce further error by transforming raw skinfold data into estimates of fat mass or percentage body fat. Thus, despite the advancement in physique assessment techniques and the notable desire of many athletes wishing to know their “body fat percentage,” the conclusions of Johnston¹³¹ remain true to this date: Practitioners are better off continuing to using raw anthropometric data than attempting to make estimates of whole-body composition from available equations.

Although the sum of skinfolds is highly correlated with body fat percentage, FFM correlates poorly with skinfolds.¹³² It has been proposed that combining skinfolds with certain body circumferences leads to a better estimate of FFM.¹³³ In theory, skinfold-corrected circumferences offer a more direct assessment of muscle mass, assuming that the skinfold thickness accurately partitions fat and lean components

at a specific site.¹³⁴ However, the skinfold-corrected girth estimates have been shown to be less accurate in monitoring changes in muscle mass than predictions using skinfolds alone.^{125,135} This imprecision may be explained, at least in part, by the fact that muscle hypertrophy does not occur uniformly throughout each body region,¹³⁶ yet the anthropometric fractionation estimate of muscle mass places equal weighting on each of five girth measurements.¹³⁷

A novel approach of assessing lean mass changes in elite athletes using a simple field test of basic anthropometric measures has recently been proposed.¹³⁸ The Lean Mass Index (LMI) is an empirical measure that tracks within-subject proportional changes in body mass adjusted for changes in skinfold thickness. As such, the LMI tracks changes in body mass not associated with changes in skinfolds. Preliminary data indicates the LMI tracks changes in FFM as well as other more time-consuming anthropometry-derived measures.¹³⁹

Aside from the convenience of surface anthropometry for assessing physique traits of athletes, parameters such as skinfolds are very robust, not readily influenced by factors such as hydration status of the athlete.¹⁴⁰ However, an interpretation of body composition using surface anthropometry is typically undertaken in conjunction with a measure of body mass (Table 3.3), and body mass can be acutely influenced by an array of factors, independent of changes in FM or skeletal muscle mass. As such, body-mass measurements should be made at the same time of day (preferably before breakfast or training but after voiding the bladder and bowel) and wearing minimal clothing,¹⁴¹ so as to minimize the influence of factors other than body composition that can impact on body mass. Other issues to consider include consistency in scales used,¹⁴² menstrual cycle phase in females,¹⁴³ and hydration status.

Precise assessment of anthropometric traits—in particular, skinfold thickness—can be difficult and therefore extreme care in site location and measurement is required if meaningful results are to be obtained. Prior to assessment, the tester should develop the appropriate technique, reducing the level of error in repeated measurements, and thus enhancing the ability to detect small but potentially important

TABLE 3.3
Interpretation of Changes in Physique Traits Based on Skinfold and Body Mass Data

Anthropometric Trait		Interpretation—Physique Trait	
Body Mass	Skinfolds	Muscle Mass	Body Fat
Increase	Stable	Gain	No change
Decrease	Stable	Loss	No change
Stable	Increase	Loss	Gain
Stable	Decrease	Gain	Loss
Increase	Increase	Potential Gain	Gain
Increase	Decrease	Gain	Loss
Decrease	Increase	Loss	Gain
Decrease	Decrease	Potential Loss	Loss

changes. The standard skinfold assessment protocol of the International Society for the Advancement of Kinanthropometry (ISAK)¹⁴⁴ follows:

- The right side of the body is used for unilateral measurements, irrespective of the preferred side of the subject, unless impractical to use due to injury or similar cause.
- Prior to measurement, ensure the skinfold caliper is accurately measuring the distance between the centers of its contact faces by using the short blades of an engineer's vernier caliper.
- The skinfold site should be carefully located using the correct anatomical landmarks.
- The skinfold is picked up at the marked site. The near edge of the thumb and index finger are in line with the marked site. The back of the hand should be facing the measurer. It should be grasped and lifted so that a double fold of skin plus the underlying subcutaneous adipose tissue is held between the thumb and index finger of the left hand. The size of the fold to pick up should be the minimum necessary to ensure that the two skin surfaces of the fold are parallel.
- The nearest edge of the contact faces of the caliper is applied 1 cm away from the edge of the thumb and finger. As a guide, the center of the caliper faces should be placed at a depth of approximately mid-fingernail.
- The caliper is held at 90° to the surface of the skinfold site at all times. The hand grasping the skin remains holding the fold the whole time the caliper is in contact with the skin.
- Measurement is recorded two seconds after the full pressure of the caliper is applied.
- Skinfold sites should be measured in succession, reducing the effects of skinfold compressibility and measurer bias.
- Duplicate or triplicate measurements should be taken where possible.

Professionals wishing to monitor the physique traits of athletes using surface anthropometry are strongly encouraged to undertake professional training. ISAK regularly offers surface anthropometry training courses internationally across a range of techniques including skinfolds, girths, breadths, and lengths. These courses are promoted on the official ISAK Web site (www.isakonline.com/).

3.8 SPECIFIED LANDMARKS FOR THE ASSESSMENT OF SKINFOLDS*

In recognition of the need for standardized methods in the assessment of skinfolds and other surface anthropometry techniques, ISAK has established clearly defined landmarks from which skinfold sites are identified. The definition of these landmarks

* From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.

and associated images to assist in their identification follow with the permission of ISAK. Technical issues such as subject positioning for each skinfold measurement are also addressed.

INTERNATIONAL SOCIETY FOR THE ADVANCEMENT OF KINANTHROPOMETRY SPECIFIED LANDMARKS FOR THE ASSESSMENT OF SKINFOLDS

Acromiale

Definition: The point on the superior aspect of the most lateral part of the acromion border.

Subject position: The subject assumes a relaxed position with the arm hanging by the side. The shoulder girdle should be in a midposition.

Location: Standing behind and on the right-hand side of the subject, palpate along the spine of the scapula to the corner of the acromion. This represents the start of the lateral border, which usually runs anteriorly, slightly superiorly, and medially. Apply the straight edge of a pencil to the lateral and superior margin of the acromion to confirm the location of the most lateral part of the border. Mark this most lateral aspect. The acromion has an associated bone thickness. Palpate superiorly to the top margin of the acromion border in line with the most lateral aspect.

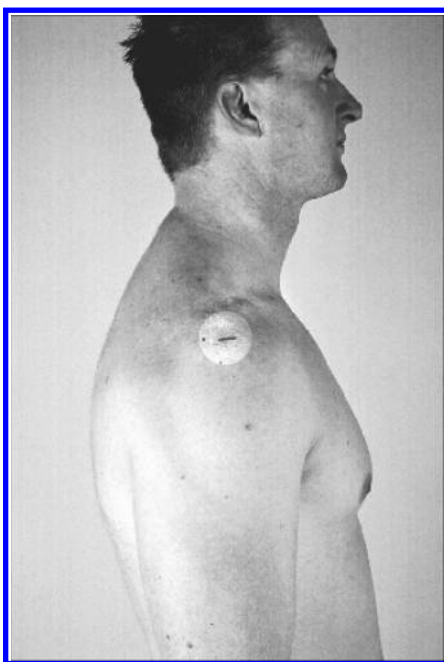


FIGURE 3.1 Acromiale landmark. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)



FIGURE 3.2 Radiale landmark. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

Radiale

Definition: The point at the proximal and lateral border of the head of the radius.

Subject position: The subject assumes a relaxed position with the arm hanging by the side and the hand in the midprone position.

Location: Palpate downward into the lateral dimple of the right elbow. It should be possible to feel the space between the capitulum of the humerus and the head of the radius. Then move the thumb distally onto the most lateral part of the proximal radial head. Correct location can be checked by slight rotation of the forearm, which causes the head of the radius to rotate.

Mid-Acromiale–Radiale

Definition: The midpoint of the straight line joining the acromiale and the radiale.

Subject position: The subject assumes a relaxed position with the arms hanging by the sides.

Location: Measure the linear distance between the acromiale and radiale landmarks with the arm relaxed and extended by the side. The best way to

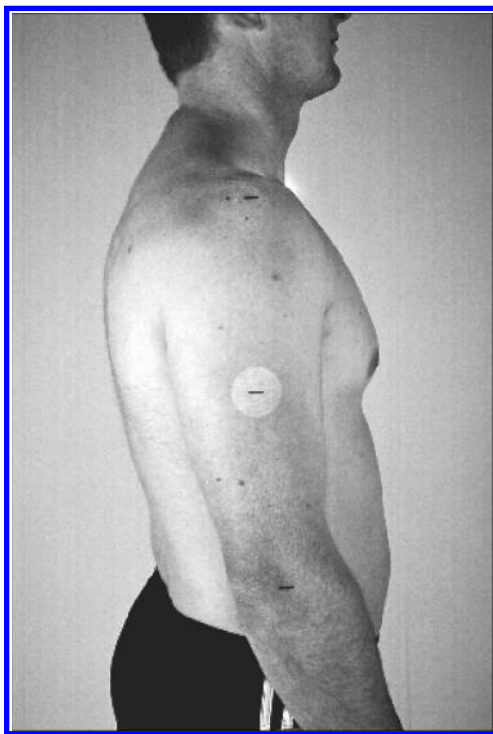


FIGURE 3.3 Mid-acromiale–radiale landmark. The other marks are the acromiale and radiale sites. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

measure this is with a segmometer or large sliding caliper. It is not acceptable to follow the curvature of the surface of the arm. If a tape must be used, be sure to hold it so that the perpendicular distance between the two landmarks is measured. Place a small mark at the level of the midpoint between these two landmarks. Project this mark around to the posterior and anterior surfaces of the arm as a horizontal line. This is required for locating the triceps and biceps skinfold sites.

Triceps Skinfold Site

Definition: The point on the posterior surface of the arm, in the midline, at the level of the marked mid-acromiale–radiale landmark.

Subject position: The subject assumes a relaxed standing position with the arm hanging by the side and the hand in the midprone position.

Location: This point is located by projecting the mid-acromiale–radiale site perpendicularly to the long axis of the arm around to the back of the arm and intersecting the projected line with a vertical line in the middle of the arm when viewed from behind.

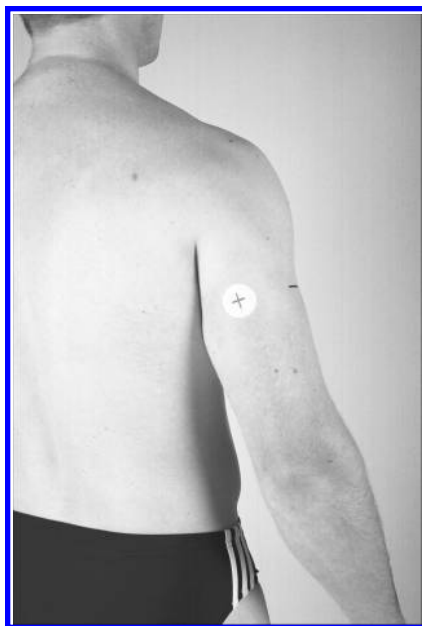


FIGURE 3.4 Triceps skinfold site. The horizontal line to the right is the marked mid-acromiale–radiale site. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

Biceps Skinfold Site

Definition: The point on the anterior surface of the arm in the midline at the level of the mid-acromiale–radiale landmark.

Subject position: The subject assumes a relaxed standing position with the arm hanging by the side and the hand in the midprone position.

Location: This point can be located by projecting the mid-acromiale–radiale site perpendicularly to the long axis of the arm around to the front of the arm and intersecting the projected line with a vertical line in the middle of the arm when viewed from the front.

Subscapulare

Definition: The undermost tip of the inferior angle of the scapula.

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides.

Location: Palpate the inferior angle of the scapula with the left thumb. If there is difficulty locating the inferior angle of the scapula, have the subject slowly reach behind the back with the right arm. The inferior angle of the scapula should then be felt continuously as the hand is again placed by the side of the body. A final check of this landmark should be made with the hand by the side in the relaxed position.



FIGURE 3.5 Biceps skinfold site. Note the marked mid-acromiale–radiale site to the left. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

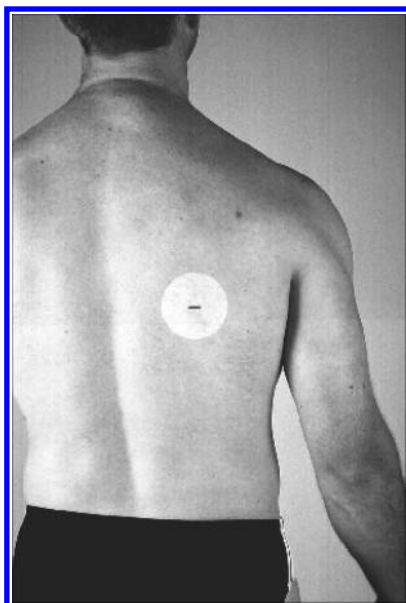


FIGURE 3.6 Subscapular landmark. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

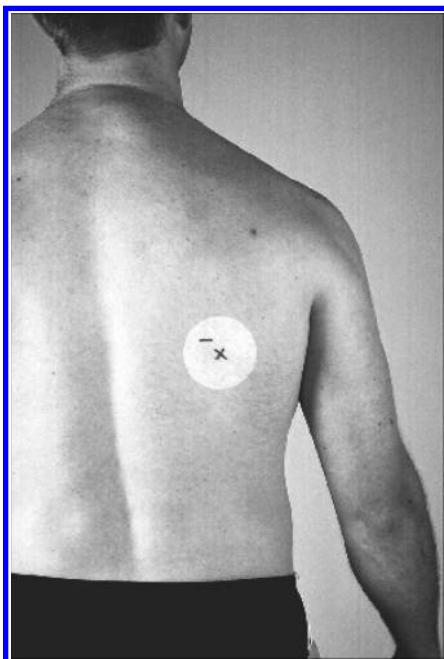


FIGURE 3.7 Subscapular skinfold site. The line (–) to the left and above is the marked subscapular site. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

Subscapular Skinfold Site

Definition: The site 2 cm along a line running laterally and obliquely downward from the subscapular landmark at a 45° angle.

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides.

Location: Use a tape measure to locate the point 2 cm from the subscapular in a line 45° laterally downward.

Iliocristale

Definition: The point on the iliac crest where a line drawn from the mid-axilla (middle of the armpit), on the longitudinal axis of the body, meets the ilium.

Subject position: The subject assumes a relaxed position with the left arm hanging by the side and the right arm folded across the chest.

Location: Use your left hand to stabilize the body by providing resistance on the left side of the pelvis. Find the general location of the top of the iliac crest with the palm or the fingers of the right hand. Once the general position has been located, find the specific edge of the crest by horizontal palpation with the tips of the fingers. Once identified, draw a horizontal line

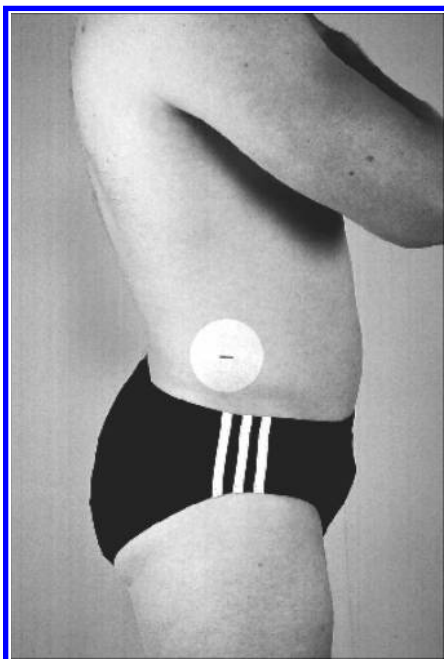


FIGURE 3.8 Iliocristale landmark. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

at the level of the iliac crest. Draw an imaginary line from the mid-axilla down the midline of the body. The landmark is at the intersection of the two lines.

Iliac Crest Skinfold Site

Definition: The site at the center of the skinfold raised immediately above the marked iliocristale.

Subject position: The subject assumes a relaxed position with the right arm folded across the chest.

Location: This skinfold is raised superior to the iliocristale. To do this, place the left thumb tip on the marked iliocristale site and raise the skinfold between the thumb and index finger of the left hand. Once the skinfold has been raised, mark its center with a cross (+). The fold runs slightly downwards anteriorly as determined by the natural fold of the skin.

Iliospinale

Definition: The most inferior or undermost part of the tip of the anterior superior iliac spine.

Subject position: The subject assumes a relaxed position with the right arm folded across the chest.

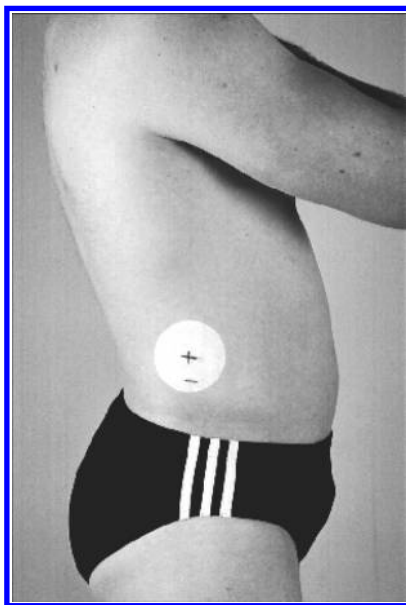


FIGURE 3.9 Iliac crest skinfold site. The lower line (–) is the marked iliocristale site. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)



FIGURE 3.10 Iliospinale landmark. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

Location: Palpate the superior aspect of the ilium and follow it anteriorly until the anterior superior iliac spine is reached. The landmark is marked at the lower margin or edge where the bone can just be felt. Difficulty in appraising the landmark can be eased by the subject lifting the heel of the right foot and rotating the femur outward. Because the sartorius muscle originates at the iliospinale, this movement of the femur enables palpation of the muscle and tracing to its origin.

Note: On females, the landmark is usually proportionally lower on the trunk, due to the flatter and broader shape of the female pelvis.

Supraspinale Skinfold Site

Definition: The point at the intersection of two lines:

1. The line from the marked iliospinale to the anterior axillary border, and
2. The horizontal line at the level of the marked iliocristale.

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides. The right arm may be abducted after the anterior axillary border has been identified.

Location: Run a tape from the anterior axillary border to the marked iliospinale, and draw a short line along the side roughly at the level of the iliocristale. Then

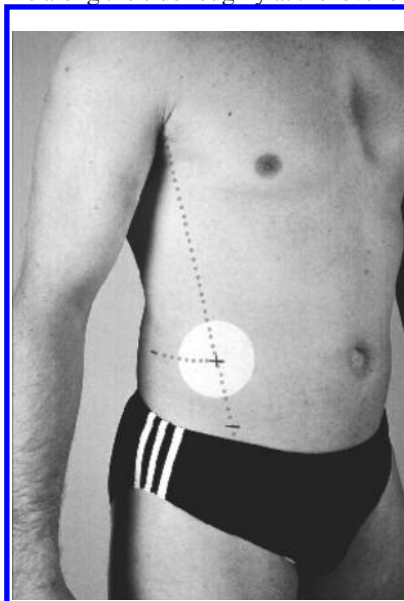


FIGURE 3.11 Supraspinale skinfold site. The dotted line from the marked iliospinale to the anterior axillary border and the horizontal line at the level of the marked iliocristale is for illustrative purposes only. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

run the tape horizontally around from the marked iliochrastale to intersect the first line.

Abdominal Skinfold Site

Definition: The point 5 cm horizontally to the right-hand side of the omphalion (midpoint of the navel).

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides.

Location: The site is identified by a horizontal measure of 5 cm, to the subject's right, from the omphalion. The skinfold taken at this site is a vertical fold.

Note: The distance of 5 cm assumes an adult height of approximately 170 cm.

Where height differs markedly from this, the distance should be scaled for height. For example, if the stature is 120 cm, the distance will be $5 \times 120/170 = 3.5$ cm.

Medial Calf Skinfold Site

Definition: The point on the most medial aspect of the calf at the level of the maximal girth.

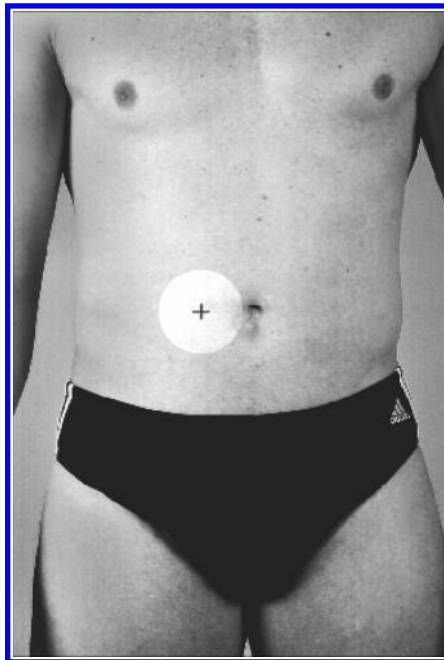


FIGURE 3.12 Abdominal skinfold site. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)



FIGURE 3.13 Medial calf skinfold site. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides. The subject's feet should be separated with the weight evenly distributed.

Location: The level of the maximum girth is determined by trial and error. It is found by using the middle fingers to manipulate the position of the tape in a series of up or down measurements. Once the maximal level is located, the point is marked on the medial aspect of the calf with a small cross (+) or other suitable mark.

Note: For easier viewing, the photograph shows the medial aspect of the lower leg. However, the site is located with the subject standing.

Front Thigh Skinfold Site

Definition: The midpoint of the linear distance between the inguinal point and the patellare (the midpoint of the posterior, superior border of the patella).

Subject position: The subject assumes a seated position with the torso erect and the arms hanging by the sides. The knee of the right leg should be bent at a right angle.

Location: The measurer stands facing the right side of the seated subject on the lateral side of the thigh. If there is difficulty locating the inguinal fold, the subject should flex the hip to make a fold. Place a small horizontal mark at the level of the midpoint between the two landmarks. Now draw a



FIGURE 3.14 Front thigh skinfold site. (From Marfell-Jones, M., Olds, T., Stewart, A., and Carter, J.E.L., *International Standards for Anthropometric Assessment*, © 2007. Used with permission of the International Society for the Advancement of Kinanthropometry.)

perpendicular line to intersect the horizontal line. This perpendicular line is located in the midline of the thigh. If a tape is used, be sure to avoid following the curvature of the surface of the skin.

INTERNATIONAL SOCIETY FOR THE ADVANCEMENT OF KINANTHROPOMETRY SPECIFIED SKINFOLD MEASUREMENTS

Triceps

Definition: The skinfold measurement taken parallel to the long axis of the arm at the triceps skinfold site.

Subject position: The subject assumes a relaxed standing position with the right arm hanging by the side and the hand in the midprone position.

Subscapular

Definition: The skinfold measurement taken with the fold running obliquely downward at the subscapular skinfold site.

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides.

Method: The line of the skinfold is determined by the natural fold lines of the skin.

Biceps

Definition: The skinfold measurement taken parallel to the long axis of the arm at the biceps skinfold site.

Subject position: The subject assumes a relaxed standing position with the right arm hanging by the side and the hand in the midprone position.

Iliac Crest

Definition: The skinfold measurement taken near horizontally at the iliac crest skinfold site.

Subject position: The subject assumes a relaxed standing position. The right arm should be either abducted or placed across the trunk.

Method: The line of the skinfold generally runs slightly downward posterior-anterior, as determined by the natural fold lines of the skin.

Supraspinale

Definition: The skinfold measurement taken with the fold running obliquely and medially downward at the supraspinale skinfold site.

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides.

Method: The fold runs medially downward and anteriorly at about a 45° angle as determined by the natural fold of the skin.

Abdominal

Definition: The skinfold measurement taken vertically at the abdominal skinfold site.

Subject position: The subject assumes a relaxed standing position with the arms hanging by the sides.

Method: It is particularly important at this site that the measurer is sure the initial grasp is firm and broad since often the underlying musculature is poorly developed. This may result in an underestimation of the thickness of the subcutaneous layer of tissue. (Note: Do not place the fingers or caliper inside the navel.)

Front Thigh

Definition: The skinfold measurement taken parallel to the long axis of the thigh at the front thigh skinfold site.

Subject position: The subject assumes a seated position at the front edge of the box with the torso erect, the arms supporting the hamstrings and the leg extended.

Method: Because of difficulties with this skinfold, two methods are recommended. Be sure to record on the *pro forma* the method used as A or B. In both methods, the leg is extended, and the subject supports the hamstrings.

Method A: The measurer stands facing the right side of the subject on the lateral side of the thigh. The skinfold is raised at the marked site, and the measurement taken.

Method B: Subjects with particularly tight skinfolds are asked to assist by lifting the underside of the thigh (as in Method A). The recorder (standing on the subject's left) assists by raising the fold, with both hands, at about 6 cm either side of the landmark. The measurer then raises the skinfold at the marked site and takes the measurement.

Medial Calf

Definition: The skinfold measurement taken vertically at the medial calf skinfold site.

Subject position: The subject assumes a relaxed standing position with the right foot placed on the box. The right knee is bent at about 90°.

Method: The subject's right foot is placed on a box with the calf relaxed. The fold is parallel to the long axis of the leg.

3.9 ULTRASOUND

Ultrasound has been used as an alternative, noninvasive method to surface anthropometry to measure subcutaneous adipose tissue (SAT). Use of ultrasound for measurement of subcutaneous fat was proposed to overcome some of the drawbacks of subcutaneous skinfolds, particularly error associated with the compressibility and elasticity of skinfolds,¹⁴⁵ accurate measurement in the obese,¹⁴⁶ and measurement of SAT at sites that are difficult for skinfold callipers.¹⁴⁷ Ultrasound is capable of measuring subcutaneous fat to a depth of approximately 100 mm or more and emerged as a body-composition tool in the late 1960s. Although initial research was promising with respect to precision and accuracy compared to skinfolds, over the years this application has not kept pace with the developments in ultrasound technology. The recent availability of higher resolution, portable, and more affordable ultrasound equipment has created renewed interest in this technology for body-composition assessment in field settings.

Ultrasound uses high-frequency sound waves introduced to the skin by means of a probe applied to the skin surface together with ultrasound gel. The sound waves are reflected back to the probe mainly from the deep fascia tissue where they are converted into an electrical signal. Ultrasound constructs cross-sectional images from reflected sound waves and can measure thicknesses of subcutaneous adipose tissue and muscle, muscle cross-sectional area, and abdominal depth. Images can be “frozen” to allow application of electronic calipers to measure areas or depths to the nearest 1 mm. Ultrasound measurement of subcutaneous adipose tissue avoids the error associated with skinfold compression from external caliper use. Measures can also be made in obese subjects where caliper application can be difficult.¹⁴⁸

Research using ultrasound for measurement of intra-abdominal adipose tissue in overweight subjects has reported a strong correlation ($r = 0.08$) between ultrasound and computed tomography (CT).¹⁴⁹ Fanelli et al.¹⁵⁰ also reported a significant

correlation ($r = -0.58$ to -0.70) between body density determined by hydrodensitometry and subcutaneous adipose tissue via ultrasound. A recent study in wrestlers¹⁵¹ demonstrated similar estimates of FFM when subjects were measured by ultrasound and hydrodensitometry. Another study in a mixed athlete population reported strong correlations for both women ($r = 0.97$) and men ($r = 0.98$) when body fat estimates measured by ultrasound were compared with DXA.¹⁵² Ultrasound may also be useful for tracking changes in muscle atrophy or restitution as a result of injury or training.¹⁵³ Despite these recent promising results, limited validation work on ultrasound renders this method as predominantly a research tool at this stage, but with further validation this approach may be an alternative to skinfold anthropometry for measurement of subcutaneous adipose tissue and estimation of FFM.

Recent development of more affordable, portable ultrasound devices that require minimal technical expertise and produce automated, instantaneous results on body composition have opened up this approach to field-based athlete assessment.¹⁵² The method is safe and not associated with any health risks. Although more studies are beginning to appear in the literature, additional validation work is clearly needed. It remains to be seen if this method will in the long term be more cost effective, practical, and useful than existing skinfold anthropometry.

3.10 THREE- AND FOUR-COMPARTMENT MODELS

A number of well-accepted two-compartment (FM and FFM), body-composition assessment models are potentially available to monitor body composition, including hydrodensitometry, air displacement plethysmography, and deuterium dilution. These methods are based on the premise that the body can be separated into two chemically distinct compartments—that is, FM and FFM.²⁹ However, each of these methods carries with it some degree of error, most of which lie not in the technical accuracy of the measurements but in the biological variability of the assumptions associated with each technique in the generation of body-composition data from raw measures like body density and total body water. This is especially the case for FFM estimates. The combination of data from several of these two-compartment models into a multicompartment model reduces the number of assumptions made and is now recognized as the current “gold standard” in body-composition assessment.

A commonly used three-compartment model approach adjusts the body density obtained from hydrodensitometry or air displacement plethysmography¹⁵⁴ for FFM hydration or total body water using isotope dilution, rather than assume a FFM total-body water content of 73.72%.²² Variation in FFM hydration away from the assumed constant, as occurs in states of hypohydration and hyperhydration respectively, increase and decrease FFM density with associated under- and overestimation of percentage body fat via hydrodensitometry by as much as 10%.³² Furthermore, measurement of FFM hydration is particularly relevant given that it has by far the lowest density of any of FFM component yet occupies the largest percentage of the FFM.

The introduction of DXA has afforded the creation of a four-compartment model, controlling for biological variability in both total body water and bone mineral content. While this model is theoretically more valid than the three-compartment model because it controls for biological variability in both bone mineral content and total body water,

work by Withers and associates³⁰ indicates the additional control for interindividual variation in bone mineral mass achieves little extra accuracy, at least in young, untrained and trained males and females. This supports the original work of Siri,²⁴ which indicates that the largest source of measurement error is related to FFM hydration.

While this multicompartiment approach to body-composition assessment can be time consuming and expensive, it is now widely recognized as the gold standard in body-composition assessment, and thus is recommended when undertaking research where changes in body composition are a key outcome measure. It should also be the criterion against which other body-composition assessment techniques are validated.

3.11 NEW TECHNOLOGIES

3.11.1 COMPUTED TOMOGRAPHY

CT is a radiological technique first used for brain imaging in the early 1970s¹⁵⁵ and has been used since around 1979 for the study of body composition.¹⁵⁶ CT scanners provide high-resolution cross-sectional images through any region of the body. Clear anatomical boundaries can be delineated between adipose tissue, skeletal muscle, visceral organs, brain, and bone. Recognition of the value of CT for measurement of regional adipose tissue followed after the work of Borkan et al.,¹⁵⁷ which reported on age-related differences in adipose tissue distribution. Two other groups subsequently reported on different approaches to measurement of whole body and regional adipose tissue measurement.^{158,159} The link between obesity-related comorbidity and visceral adipose tissue¹⁶⁰ has driven increased use of CT for measurement of body composition.

CT images for body composition are built up of pixels that have a CT or HU (Hounsfield unit) number. The CT number is a measure of attenuation relative to water (HU = 0) and air (HU = -1000). CT uses the different attenuation characteristics of tissues (based on different chemical composition and density) to determine composition. Generally, when measuring the fat content of skeletal muscle, the lower the HU value, the lower the density and the greater proportion of fat.¹⁶¹ Specific information relating to the more radiographic, technical aspects of CT are beyond the scope of this chapter but those interested would find the following references useful.^{156,162,163}

Rössner et al. completed one of the earlier CT validation studies for adipose tissue cross-sectional area via comparison with cadavers.¹⁶⁴ High correlation coefficients were found between CT and planimetry for both total ($r = 0.94$) and intra-abdominal ($r = 0.83$) adipose tissue area. A number of subsequent studies in humans and animals, including comparison using chemical extraction of fat,¹⁶⁵ support that CT is accurate and precise and that repeatability is high.¹⁶⁶ In non-athlete populations, selected CT scan volumes of adipose tissue have been highly correlated with total FM in men and women.^{167–169} CT is often used as a reference technique for validation of other body-composition approaches.¹⁶¹ Technological advances since these studies were undertaken have improved the resolution of images and extended the measurement to vascular and bone tissue.¹⁵⁶

A major impediment to the use of CT for body-composition measurement is radiation exposure (abdominal CT results in an effective dose in the order of 10 mSv but depends on the scanner and may range from 3 to 14 mSv),¹⁷⁰ and this limits its use for

whole-body or longitudinal studies in humans.¹⁵⁶ Use for nonclinical purposes such as body-composition monitoring in healthy athletes, children, adolescents, and child-bearing (or pregnant) women is also contraindicated, and this would still be the case even with the availability of newer low-dose approaches available in some laboratories.¹⁷¹ The more common need to assess whole-body composition, radiation exposure, and significant cost of CT renders this a method that only has application for specific regional body-composition research. The introduction of magnetic resonance imaging (MRI), which eliminates exposure to radiation, is now more typically used in athletes, although again the cost and relatively limited availability of this method means it also is predominantly a research tool for body-composition assessment.

3.11.2 MAGNETIC RESONANCE

Measurement of body composition by MRI requires the subject to be placed inside an imaging device that then creates a magnetic field approximately 10,000 times stronger than the outside or natural earth force.¹⁶¹ Proton MRI, most commonly used for clinical and body-composition analysis, is based on the interaction between the nuclei of hydrogen atoms, which are prolific in all biological tissues. Protons or hydrogen nuclei behave like small magnets and when exposed to the imaging device, the protons align with the created magnetic field. A pulsed radiofrequency field (RF) can be applied to the subject within the magnet and this causes many of the protons to either flip or absorb energy. When the RF is removed, the protons gradually release the absorbed energy and generate a specific RF signal that can be used to create images on a computer. Different tissues such as muscle, fat, and bone have differential proton density and release absorbed energy at different rates. The RF pulse parameters can be manipulated to exploit the difference in proton density and energy release times between tissues such as fat and muscle to create high-quality images.¹⁶¹

Application of MRI for body composition was first done by Foster et al.¹⁷² and Hayes et al.¹⁷³ who quantified subcutaneous adipose tissue using MRI. Many studies since have used MRI to assess skeletal and adipose tissue area and distribution in clinical and normal populations.¹⁷⁴ Many of these studies have used a single cross-sectional image but unlike CT, multiple images can be collected using MRI without the health risk of increased radiation dose associated with CT. Technological improvement has significantly reduced the time taken to capture images, and it is now possible to obtain whole-body MRI data in approximately 30 min.¹⁶¹

Rapid advances in technology have also extended the application of this methodology beyond measurement of body fat and lean tissue. Functional MRI can be used to track the metabolic activity of organs and tissues and this, in addition to body-composition measurements, can be used to more deeply explore the underpinning mechanisms associated with disease, especially obesity-related comorbidities. Diffusion tensor imaging can be used for assessing muscle fiber type and proton (¹H-MRS) and phosphorus (³¹P-MRS) spectroscopy for water-fat imaging. Sodium MRI and whole-body nuclear MRI systems are also available to quantify total body lipid and water.^{156,163} To date, only one validation study exists for nuclear quantitative magnetic resonance (QMR) via comparison to a four-compartment model. This methodology uses the differences in resonance of organic and inorganic hydrogen

to fractionate signals from fat, lean tissue, and water. QMR underestimated fat and overestimated lean mass, and this error increased with increasing adiposity.¹⁷⁵ However, the method is simple and rapid and can be completed in less than 3 min, and further validation work will hopefully bring better understanding and refinement of its accuracy and precision.¹⁶³

Imaging methods such as MRI and CT are considered to be among the most accurate for *in vivo* measurement of body composition. High correlation between MRI and CT for abdominal subcutaneous adipose tissue (0.79), visceral adipose tissue (0.79), and total adipose tissue (0.99) are observed¹⁷⁶ and coefficient of variation is low (~1.5) for whole body¹⁷⁷ and for total abdominal adipose tissue (3%).^{161,178}

As there is no exposure to radiation, it is feasible that MRI has application for longitudinal monitoring of athletes that can be extended to children and adolescents. The major limitation with this technology is the size of the person that can be measured (as with CT, persons with a BMI > 40 kg.m⁻² do not fit inside the scanner), high cost, and limited availability of scanner time, which is typically heavily saturated with a clinical diagnostic load. Scanning is also contraindicated for individuals who are claustrophobic or have metal plates, pins, or pacemakers inserted. In athlete groups, the main contraindication is claustrophobia, large body mass (for example, U.S. football players) or stature, and existence of internal metal plates or pins. MRI therefore remains a research-only method for body-composition analysis.¹⁶³

3.11.3 THREE-DIMENSIONAL PHOTONIC SCANNING

Three-dimensional photonic scanning (3DPS) provides information on the three-dimensional size and surface area of the body, and the approach generates data on regional body volume and dimensions. The application was initially developed for use in the clothing industry to capture information about body-surface topography, but soon after it was recognized that the technology has potential application for medical¹⁷⁹ and sports performance fields.¹⁸⁰ In brief, subjects being measured for anthropometric or body-composition assessment wear tight underwear or swimwear and adopt a standardized position during scanning. Standard anatomical landmarks using reflective markers are needed for measurement of body dimensions. The scanner projects stripes of safe white light onto the body and captures the light distortion via use of stationery cameras over a period of seconds. The scanners can accommodate individuals up to 2.1 m in height and 1 m in depth. The cluster of photonic data points are used to reconstruct skin topography using computer algorithms.^{180–182} The data can be used to calculate total and regional body volumes and surface area, and can automatically calculate lengths and dimensions. Body volume can be used to calculate density and body composition.

The method is still relatively new but it is time efficient, noninvasive, and suitable for serial measurements. It has been used in a large study investigating the relationship between shape and BMI and to examine associations between gender and size in the U.K. National Sizing Study. This study showed that BMI was significantly associated with hip and bust in women, and chest and waist size in men. Validation work on this approach for measurement of body composition is limited,^{179,183} but a recent study by Wang et al.¹⁸³ compared 3DPS with hydrodensitometry and tape

measurements for the assessment of body volume, circumferences, lengths, and percentage body fat. The data generated by the scanner for body volume and lengths was greater than UWW and tape-derived lengths, respectively, but the values for percentage body fat were similar. Further validation work is required before the technique could be confidently used for measurement of body composition in a variety of populations, including athletes.

The 3DPS approach is safe and can be used in children and adults with no known contraindications. It has application in epidemiology to investigate the association between shape and health, and within sport, there is a known association between shape and athletic performance,¹⁸⁰ which could be more specifically detailed by this approach. The technology is currently expensive and not easily portable so the technique is not practical for field use and is only used for research at this stage. However, its safety and the potential for individuals to use this more widely in the community for sizing applications may help this technology to rapidly develop and proliferate, making measurement more available and affordable for athlete monitoring in the not-too-distant future. The world's first anthropometric survey using 3D scanning took place at the 2007 Australian Rowing Championships.¹⁸⁴

3.12 PRACTICAL RECOMMENDATIONS

A wide range of tools are available to assess the physique traits of athletes. Aside from talent identification initiatives and the identification of a preferred physique for a given sport and player position, the primary focus is given to the longitudinal monitoring of physique traits. As such, techniques that are cost and time effective, portable, reliable, and safe and provide insight into all physique traits, including both fat and muscle mass, are a priority. Given this, surface anthropometry remains an effective tool within athletic populations, but it demands highly skilled technicians if reliable data are to be collected. Recognized training through organizations like ISAK is strongly recommended for anyone wishing to make use of surface anthropometry to monitor physique traits of athletes. Within Australia, the routine monitoring of body composition among athletes across regional and national institutes of sport remains with the measurement of skinfolds (\pm girths) at several anatomically identified sites on the body (see [Section 3.8](#), Specified Landmarks for the Assessment of Skinfolds).

Irrespective of the test chosen, all physique assessment tools carry with them some degree of assumptions and measurement error. Having an appreciation of this measurement error helps to distinguish between measurement error and real changes in body composition, that is, documented change in physique traits being greater than reported measurement error. Issues associated with equipment contributing to measurement error are often beyond the control of technicians, but athlete presentation can also contribute to the error of repeat assessments. As such, factors such as time of day, hydration status, and gastrointestinal tract contents should be standardized wherever possible; fasted early morning assessments may be the most reliable where practical. Minimizing the error or noise associated with a test enhances its reliability, making it easier to identify small but potentially important changes. Reliability of measurement also influences the frequency of assessment. In general,

physique assessments should not be undertaken any more regularly than every 4–8 weeks, depending on the individual athlete and his or her body-composition goals.

When collecting data, the physical and emotional well-being of the athlete should remain a priority. Sensitivity should be shown to cultural beliefs and tradition. Procedures should be explained to those unfamiliar with the testing, with information provided in advance on what testing is to be undertaken, the reason for profiling, what measurements are to be taken, and any specific requirements such as clothing to be worn. Where appropriate, consideration should be given to gender comparability between the technician and athlete, with privacy in data collection and reporting always ensured. With this in mind, consideration should be given to the establishment of electronic databases that not only provide a secure means of data collection but also automate reports that provide invaluable historical data as well as interpretation of existing results against previous assessments. Finally, where resources permit, the collection of data in duplicate should be considered, enhancing the reliability of measurement.

3.13 FUTURE RESEARCH NEEDS

Although surface anthropometry does not offer a direct measure of total fat mass or FFM, its robustness in the field, convenience, and low cost ensures it remains a popular method of body-composition monitoring among athletes. Newer technologies like DXA or the combination of technologies in a three- or four-compartment model offer an opportunity to better interpret surface anthropometry data. Preferably, this should be developed around interpretation of changes in physique traits over time rather than a one-off assessment. Such longitudinal investigations also create opportunities to help better understand the association between physique traits and competitive success.

Aligned with this, developing a better understanding of factors influencing the noise or error associated with body-composition assessment tools will enable the development of protocols that afford a much greater resolution of measurement. Ultimately, this will help to create techniques and protocols that are able to detect small but potentially meaningful changes in body composition. Once established, this will also create an opportunity to have better resolution for assessing interventions (such as dietary, training) or unforeseen situations (injury, illness, or detraining, for example) proposed to influence body composition, which ultimately will have application within not only the sports environment but also the wider community.

Our understanding of the application of newer technologies like ultrasound and 3DPS are preliminary. Further exploration of tools such as these will hopefully better our understanding of how these tools can be used into the future.

3.14 CONCLUSIONS

Body composition is just one of an array of “fitness traits” that may contribute to the overall success of an athlete. As such, the association between physique traits and competitive success should not be overemphasized. However, the regular monitoring of body composition among athletic populations can offer insight into adaptations

to training and/or dietary interventions as well as optimization of physique traits for specific sports and playing positions. An array of tools is available for the measurement of body composition, the test of choice being influenced by technical issues like safety, validity, precision, and accuracy of measurement as well as practical issues such as availability, cost, portability, invasiveness, time effectiveness, and technical expertise necessary to conduct the assessment. Among athletic populations, consideration must also be given to the unique physique traits these individuals may possess, including particularly tall, broad, and muscular individuals as well as those with very low body-fat levels. Considering these factors, the routine monitoring of body composition of athletes remains with the use of surface anthropometry, although BIA, DXA, and the BOD POD are becoming more popular as their accessibility increases. Refinement of protocols such as the standardization of how subjects present for assessment and an improved awareness of the limitations of each technique will allow more informed protocols to be developed. This will offer greater insight into acute and longitudinal monitoring of body-composition changes and their importance to competitive success, as well as tailoring interventions that can assist in the appropriate manipulation of body composition. Despite the array of tools available to assess body composition, surface anthropometry remains the most practical tool at present to monitor the body composition of athletes longitudinally. However, given the ever-increasing interest in the relationship between body composition and competitive sporting success, new, more refined assessment tools with greater reliability and resolution of measurement are likely to emerge into the future.

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4 Body Composition and Gender Differences in Performance

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4.1 INTRODUCTION

As more and more women started participating in sports over the past 50 years, they embarked on a “catch-up” race for the world records. World records improved at a

faster rate for females than for males from the 1950s until the late 1980s.¹ However, since the 1990s a stable gap has been observed between male and female elite performances, though it is evident that men typically possess greater levels of strength and power, and have larger anaerobic and aerobic capacities.^{2,3}

The relatively small number of gender-specific research studies makes it difficult to elicit the physiological mechanisms responsible for these performance differences between genders. The responsible mechanisms are probably related to specific gender differences such as body composition,^{3,4} muscle characteristics,⁴⁻⁷ and hormonal influences.^{2,8-10} It seems likely that much of the reluctance to include females as subjects in research studies is related to the need to control for hormonal fluctuations due to the menstrual cycles or oral contraceptives. Over the past 10 years, exercise physiology research on gender differences has increased, but many areas require further clarification.

Both the body size and body composition are similar in boys and girls during early childhood. During late childhood, girls begin to accumulate more fat than boys, while during early adolescence, boys start to develop their fat-free mass at a higher rate than girls.¹¹ These body-composition differences between the genders are primarily determined by significant hormonal changes that occur during development. Before puberty, small amounts of the gonadotrophic hormones—follicle-stimulating hormone (FSH) and luteinizing hormone (LH)—stimulate the growth of ovaries in girls and testes in boys. However, at puberty, the increased release of these same hormones from the anterior pituitary gland leads to significant increases in ovary size and estrogen secretion in females and testes size and testosterone secretion in males.¹² In females, this increased estrogen secretion leads to a broadening of the hips, an increased rate of bone growth, and increased fat deposition, especially in the hip and thigh regions.¹³ On the other hand, males tend to possess an increased testosterone production, leading to advanced development of the musculoskeletal and cardiovascular systems.¹¹

Another important factor to consider is that during the reproductive years, when most female athletes are competing, their bodies are exposed to rhythmical fluctuations in either endogenous (menstrual cycle) or exogenous (oral contraceptives) female steroid hormones. These variations in estrogen and progesterone not only have effects on the reproductive system but also cause physiological changes outside the reproductive system. Receptors for estrogen have been found in multiple tissues, including the brain, cardiovascular system, kidney, muscle, and many others.^{14,15} How the secondary effects of estrogen and progesterone, and their interaction, affect exercise performance in females has recently started to receive more attention in research. However, this topic is complex and not well understood, thus warranting further investigation.

This chapter addresses the three main areas of exercise performance—muscle strength, anaerobic performance, and aerobic performance—in relation to gender and body composition differences. Each section addresses both differences in body composition and potential effects of male and female steroid hormones on performance.

4.2 GENDER AND STRENGTH

4.2.1 GENDER DIFFERENCES IN STRENGTH PERFORMANCE

Muscle strength is the ability of a muscle to develop maximal contractile force and is an important factor for performance in most sports. It is widely known that males possess larger absolute muscle strength than females. In a study with a large number of young men and women, Leyk and others¹⁶ reported a difference of approximately 200 N in absolute maximal handgrip strength (540.8 ± 87.1 N for men and 329.4 ± 57.7 N for women). For the lower body, Clark et al.¹⁷ reported a similar difference between genders for knee extension (905.0 ± 33.5 N for men and 722.1 ± 33.5 N for women). Further examples of the difference in muscle strength between males and females are shown in [Table 4.1](#).

A strong relationship between muscle strength and muscle mass has been widely demonstrated.^{16,18,19} The different steroid hormone profiles in males and females result in considerable differences in body composition. The average adult male has a greater muscle mass than the average female,⁴ while the average female has a higher fat percentage than the average male.^{3,7,20,21} These findings lead to the suggestion that the strength difference between males and females is largely a result of the difference in muscle mass.

Some studies have demonstrated that this gender difference in muscle strength disappears when strength is adjusted for lean body mass.^{22,23} Others, however, have reported that the difference is reduced when expressed per lean body mass but that it does not disappear.^{16,18,19} It also seems that this gender difference in strength may be dependent on muscle location, as the relative strength of the upper body muscles remains larger than for the lower body.¹⁶ Miller and colleagues¹⁹ suggested that this may be attributed to the lower portion of women's lean body mass being located in the upper body.

It is clear that muscle mass plays an important role in explaining the strength difference between genders. Other factors that may affect muscle strength are muscle architecture and neural activation.¹⁹ With regard to gender differences it is also important to consider hormonal effects, including those of the menstrual cycle, on muscle strength.

4.2.2 FACTORS INFLUENCING STRENGTH PERFORMANCE

4.2.2.1 Muscle Characteristics

Important aspects of muscle characteristics are the total number of muscle fibers, the fiber type distribution, and the size of the muscle fibers. Some studies have reported a smaller number of muscle fibers in females than males,^{24,25} while most have shown no difference in total fiber number between genders.^{19,26–28} Equivocal results have been found regarding a potential difference between males and females in fiber type distribution based on the number of muscle fibers.^{6,7,19,29,30} These conflicting findings are likely to be related to differences in subject sampling and methodology. Findings of a thorough study by Staron and others⁷ on 55 women and 95 men demonstrate

TABLE 4.1
Muscle Strength for Females and Males as Reported in Gender Comparison Studies for Different Muscle Groups

Gender	Age (yr)	<i>n</i>	Contraction	Strength	Comments	Reference
F	22 ± 1	533	Handgrip	329 ± 58 N		Leyk et al., 2007
M	21 ± 1	1654	Handgrip	541 ± 87 N		Leyk et al., 2007
F	21 ± 3	20	Handgrip	354 ± 70 N		Hunter et al., 2009
M	22 ± 5	20	Handgrip	504 ± 97 N		Hunter et al., 2009
F	25 ± 1 ^a	8	Elbow flexion	40 ^b Nm		Miller et al., 1993
M	23 ± 1 ^a	8	Elbow flexion	70 ^b Nm		Miller et al., 1993
F	26 ± 4	8	Elbow flexion	43 ± 8 Nm		Hunter et al., 2006
M	25 ± 4	9	Elbow flexion	76 ± 9 Nm		Hunter et al., 2006
F	24 ± 4	8	Dorsiflexion	18.2 ± 2.3 ^a N	Twitch force (electrical stimulation)	Russ et al., 2003
M	27 ± 4	8	Dorsiflexion	22.1 ± 2.7 ^a N	Twitch force (electrical stimulation)	Russ et al., 2003
F	24 ± 4	8	Dorsiflexion	66.8 ± 8.5 ^a N	10 Hz force (electrical stimulation)	Russ et al., 2003
M	27 ± 4	8	Dorsiflexion	83.7 ± 10.7 ^a N	10 Hz force (electrical stimulation)	Russ et al., 2003
F	24 ± 4	8	Dorsiflexion	138.5 ± 12.3 ^a N	50 Hz force (electrical stimulation)	Russ et al., 2003
M	27 ± 4	8	Dorsiflexion	195.2 ± 16.3 ^a N	50 Hz force (electrical stimulation)	Russ et al., 2003
F	25 ± 8	35	Knee extension	255 ± 51 Nm	CSA Quads 55.6 ± 6.9 cm ²	Wust et al., 2008
M	27 ± 7	29	Knee extension	339 ± 82 Nm	CSA Quads 75.5 ± 10 cm ²	Wust et al., 2008
F	25 ± 1 ^a	8	Knee extension	180 ^b Nm		Miller et al., 1993
M	23 ± 1 ^a	8	Knee extension	260 ^b Nm		Miller et al., 1993
F	21 ± 3	14	Knee extension	160 ^b Nm	F _a Type I 31 ± 5%, Fa Type II 69 ± 5%	Yasuda et al., 2005
M	21 ± 2	13	Knee extension	255 ^b Nm	F _a Type I 27 ± 3%, Fa Type II 73 ± 3%	Yasuda et al., 2005
F	24 ± 1 ^a	11	Knee extension	722 ± 34 ^a N		Clark et al., 2005
M	26 ± 2 ^a	11	Knee extension	905 ± 34 ^a N		Clark et al., 2005

Note: Data are reported as female/male pairs from the same study, ensuring similar methodology and conditions for gender comparison. Data are reported as mean values ± SD.

F_a = Fiber area.
^a = Data are reported as mean value ± SE.
^b = Value estimated.

that there is no real difference in fiber type distribution between males and females, which is supported by Yasuda et al.³¹

With regards to muscle size, women generally have a smaller total muscle cross-sectional area (CSA) than men when matched for training status.^{7,19,30,32} Staron and others⁷ found a mean total CSA of approximately 16 mm² for the vastus lateralis for young men and approximately 11 mm² for young women. Wust et al.³² used magnetic resonance imaging and reported a total CSA mean \pm SD for the quadriceps of 75.5 \pm 10 cm² for men and 55.6 \pm 6.9 cm² for women.

Even though there may not be a gender difference in fiber type distribution, the percentage area occupied by the different fiber types does appear to exhibit a gender difference. Women typically have a smaller fiber-area percentage for Type II fibers and a larger fiber-area percentage for Type I fibers.^{7,31} Staron et al.⁷ found that the fiber area percentage for Type I in the vastus lateralis was significantly larger for females than males (44.0 \pm 11.6 for women and 36.2 \pm 11.6 for men), while for Type IIA the percentage was significantly smaller for females (33.6 \pm 8.7 for women and 41.2 \pm 9.4 for men). They found no significant difference in fiber area percentage for Type IIB between genders (22.4 \pm 10.3 for women and 22.6 \pm 11.8 for men).⁷ Yasuda and others³¹ reported smaller but still significant differences in Type I fiber area percentage (31.4 \pm 4.6 for women and 27.2 \pm 3.3 for men) and in the combined Type II fiber area percentage (68.6 \pm 4.6 for women and 72.8 \pm 3.3 for men).

Type I muscle fibers have a slower contraction speed and are more fatigue resistant than Type II fibers.³³ It could therefore be suggested that the higher Type I fiber-area percentage in women may make them more suited to prolonged activity.² Several studies have shown that women can maintain submaximal muscle contractions longer than men.^{17,32,34,35} Besides fiber type composition, other potential explanations for this gender difference in muscle fatigue are neural activation and blood flow.³² Hunter et al.³⁶ used superimposed electrical stimulation to demonstrate that there are no gender differences in voluntary activation, while Wust and colleagues³² found the same result when eliminating any neural activation problems by using electrical stimulation only. These findings demonstrate that the gender difference in fatigue resistance is not related to central activation and is likely to have a peripheral cause. During repeated contractions the rate of fatigue depends on depletion of energy stores during contraction and recovery during relaxation. Aerobic recovery, in turn, will depend on blood flow and oxidative capacity of the muscle fibers.³² Wust et al.³² occluded blood flow to investigate this further and still found a gender difference in fatigue, indicating that blood flow is not a likely explanation. Others, however, found that the gender difference in muscle fatigue disappeared when muscle blood flow was occluded,^{17,37} but these studies did not use electrical stimulation to control for neural activation. As women typically have a higher fiber-area percentage for Type I fibers than men,^{7,31} it seems plausible that fiber type composition at least partly explains the gender difference in muscle fatigue.

4.2.2.2 Neural Activation

Miller and colleagues¹⁹ reported that the estimated number of motor units and the number of muscle fibers per motor unit did not differ between genders for both the vastus lateralis and the biceps brachii. Motor unit activation, calculated from the

supramaximal twitch superimposed on the maximal voluntary contraction compared to the rest measure, was similar for men and women for both biceps brachii and vastus lateralis.¹⁹ More recent studies have confirmed that there were no significant gender differences in voluntary muscle activation.^{32,36}

4.2.2.3 Hormonal Factors

Testosterone has been shown to increase muscle protein synthesis (MPS)^{38,39} and muscle mass increases when MPS exceeds muscle protein breakdown.⁴⁰ After puberty, testosterone concentrations in males remain approximately ten to fifteen times larger than in females.³¹ It therefore is not surprising that males generally have a larger muscle mass than females. However, research on gender differences in protein metabolism to support this theory is sparse.

Whole-body protein metabolism studies showed no gender difference in leucine rate of appearance (index of whole-body protein breakdown) and nonoxidative leucine disposal (index of MPS) at rest.²¹ When investigating muscle protein balance it has been observed that rates of protein synthesis and breakdown were greater in males than females at rest.⁴¹ However, when these rates were normalized for muscle mass, there was no difference between genders.^{41,42} The methodologies used in these studies combined with small subject numbers and large between-subject variability may have caused difficulties in detection of small gender differences in muscle protein metabolism. It is evident that further research in this area is needed, but the limited research to date suggests that basal muscle protein balance in adults cannot explain the gender difference in muscle mass. It could be suggested that the muscle mass difference may be largely explained by changes during puberty or there may be gender differences in response to acute anabolic stimulation.⁴²

Large fluctuations in muscle protein balance occur as a result of acute anabolic stimulation, such as muscle contraction and nutrient intake. MPS immediately following resistance exercise is decreased.^{43–45} However, at 1 and 2 hours postexercise, a significant increase in MPS is shown⁴⁴ and this effect lasts for up to 48 hours.⁴⁶ An increase in amino acid availability through nutrient intake during this 48 hours post exercise stimulates a greater MPS.^{40,43} Research into the dose-response relationship for MPS showed that an intake of 20 g of protein intake maximally stimulated MPS following resistance exercise.⁴⁷ The best timing for postexercise nutrient intake has been open to debate, but a minimum delay appears most beneficial for muscle adaptations.⁴³ It has been suggested that there is no gender difference in responses to resistance exercise⁴⁸ and nutrient intake.⁴³ Most research, however, has been conducted on males only or did not include gender comparisons. Further research on gender differences in muscle protein synthesis responses to exercise and nutrient intake is required.

Testosterone clearly has a positive effect on muscle protein synthesis, but much less is known about the effects of the ovarian hormones on protein synthesis. Most of the available evidence is based on animal and *in vitro* studies and supports an inhibiting effect of estrogen on muscle protein synthesis.⁴⁹ The effect of progesterone is inconclusive with progesterone administration in rats, showing both an increase⁵⁰ and a decrease⁴⁹ in muscle protein synthesis. Further research, with a focus on *in*

vivo studies, is needed to clarify the effect of the ovarian hormones on muscle protein synthesis.

4.2.2.4 Menstrual Cycle

Throughout ovulatory menstrual cycles, women are exposed to continuously changing female steroid hormone profiles. Miller and others⁵¹ measured muscle protein synthesis at rest and after exercise during the menstrual cycle. This first study of its kind reported no difference in muscle protein synthesis between the follicular (low estrogen and progesterone) and luteal (elevated estrogen and progesterone) phase of the menstrual cycle.⁵¹ However, a limitation of this study is its cross-sectional design (subjects were only tested once in either the follicular or luteal phase). It is evident that further research in this area is also required.

The research literature is equivocal concerning the effects of the menstrual cycle hormone fluctuations on muscle strength. Several studies have reported changes in muscle strength throughout the menstrual cycle,^{52–55} while others found no change.^{9,10,56–59} These conflicting findings can largely be attributed to methodological shortcomings.⁸ Janse de Jonge and colleagues⁵⁸ addressed these limitations through the use of superimposed electrical stimulation to ensure maximal neural activation and use of strict hormone limits to verify menstrual cycle phase. This study found no difference in skeletal muscle strength, fatiguability, or electrically evoked contractile characteristics between menstruation (low estrogen and progesterone), the late follicular estrogen peak, and the luteal phase (elevated estrogen and progesterone).⁵⁸ More recent studies have also confirmed that hormone fluctuations throughout the menstrual cycle do not affect muscle contractile characteristics.^{60,61}

In conclusion, there are obvious gender differences in muscle strength with males being stronger than females. This gender difference is clearly related to body composition, as research has shown that it is greatly reduced or even disappears when strength is expressed relative to lean body mass. Factors that may explain the remaining difference are muscle characteristics, neural activation, and hormonal influences. It has been demonstrated that muscle strength does not vary across the menstrual cycle. Most research indicates that there is no gender difference in total number of fibers and fiber type distribution. For the fiber area percentage, however, it was found that women have a larger Type I area percentage and a smaller Type II area percentage than men. It has been suggested that this difference may partly explain why women are more fatigue resistant than men. No gender difference has been observed in neural activation and in whole-body protein metabolism normalized for muscle mass. Further research on muscle protein synthesis responses to exercise and nutrient intake with a focus on gender comparisons and the menstrual cycle is needed.

4.3 GENDER AND ANAEROBIC PERFORMANCE

4.3.1 GENDER DIFFERENCES IN ANAEROBIC PERFORMANCE

Anaerobic performance corresponds to the ability to produce high levels of power during periods of very high-intensity exercise ($> 100\% \text{ VO}_{2\text{max}}$) that typically last anywhere between 10 and 100 seconds.⁶² Anaerobic energy provision occurs within

the cytoplasm of the muscle cell through the degradation of creatine phosphate stores or through anaerobic glycolysis of simple carbohydrates. This, in addition to the typically high force outputs, suggests that the limiting factors are within the working muscles or are external factors that affect muscle size and function.

From the existing literature, it is well established through both athletic performance records and scientific data that anaerobic performance is largely different between genders.^{1,63–69} The data illustrated in Figure 4.1 demonstrate the longitudinal differences in world records and Olympic best times between genders in anaerobic events. Seiler et al.¹ have reported that the anaerobic performance difference between males and females reached a minimum of 10.3% between 1976–1988 and since then, has actually increased to 11.5%. The researchers suggested that the increase from the nadir cannot be explained due to secondary factors such as participation rates

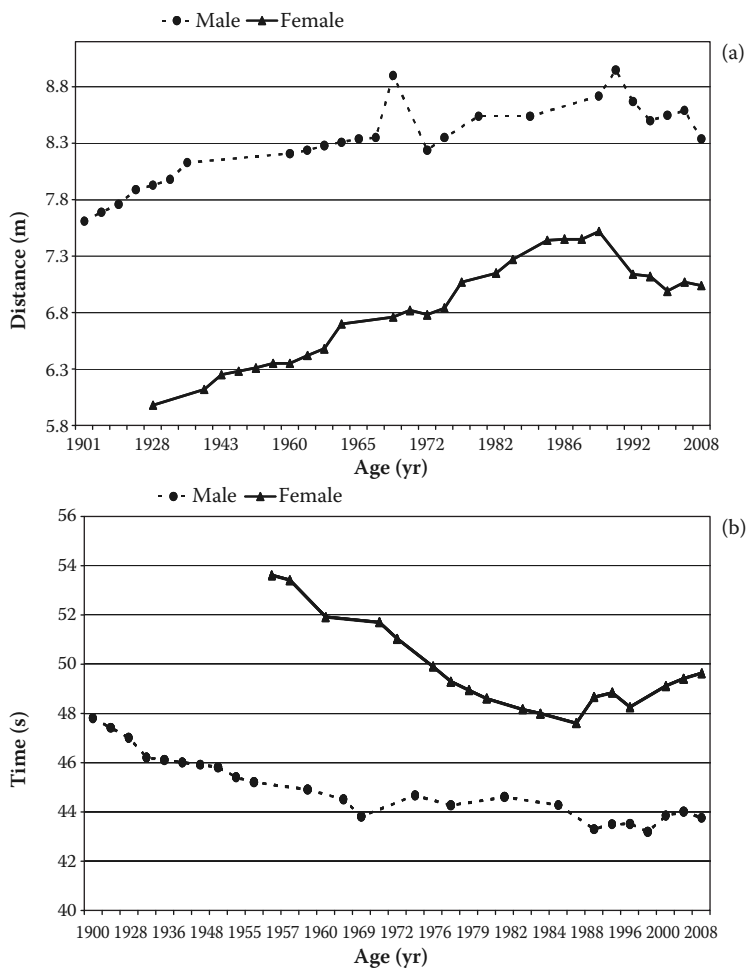


FIGURE 4.1 A gender-comparison in world-record progression and Olympic winning times in the (a) long jump; (b) 400 m track sprint.

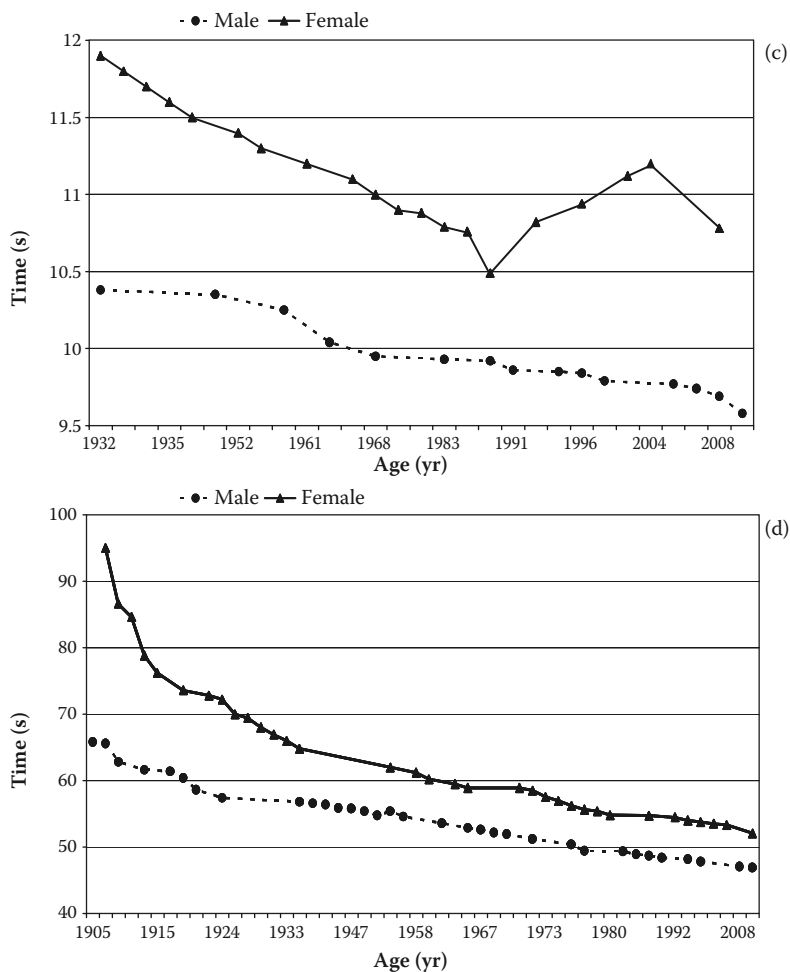


FIGURE 4.1 (continued) A gender-comparison in world-record progression and Olympic winning times in the (c) 100 m track sprint; and (d) 100 m freestyle swim.

or technological advances or to declines in the training practices of female athletes. Seiler et al.¹ suggests that the expansion of this difference most likely corresponded to the introduction of a more rigid and sensitive drug testing regime within elite sport and that the current difference may reflect the “natural” physiological difference between genders. Similarly, several scientific studies have repeatedly reported that male participants demonstrate higher anaerobic peak power and capacity than their female counterparts.^{1,65–70} These researchers help demonstrate that the observed difference between genders in competition performance also exists in laboratory-based tests for specific physiological capacities. Bouchard et al.⁶² has suggested a range of factors that have the capacity to influence anaerobic performance, and these can be broadly classified into areas such as muscle mass and function, bioenergetics, heredity, training, and gender. However, it does appear that the strongest influence on

anaerobic performance comes from muscle mass and energy metabolism in regards to the effects of body composition and gender.

4.3.2 FACTORS INFLUENCING ANAEROBIC PERFORMANCE

4.3.2.1 Muscle Mass

A wealth of research is available that strongly demonstrates the relationship between body size and muscle mass with anaerobic performance. A number of researchers have presented data reporting that the observed gender difference in anaerobic power output is completely removed when corrected for total body mass (BM) or lean body mass (LBM).^{1,63–70} The anthropometrical differences in body composition between genders are well established, with females tending to typically possess higher relative levels of body fat (%BF) and less LBM than males.^{4,20,21,71} This disparity is most likely a strong correlate to the differences observed in physiological components between genders. From the available data it appears that a gender difference is noticeable in anaerobic performance and capacity and that the typical difference reported in lean muscle mass is the most likely explanatory physiological mechanism.

With respect to performance in anaerobic events, Stefani⁶⁸ has demonstrated that the difference between genders is largely due to the reported difference in lean muscle mass in a range of elite athletic competitions (running, speed skating, jumping, swimming, and rowing). The researchers reported that the differences in estimated power output between genders in each selected event (100%, $n = 32$) and individual performances in each event (96%, $n = 411$) were within one standard deviation for the LBM percentage difference. This finding suggests that the power output and subsequent performance of male and female athletes are consistent with calculated differences in their LBM.

While the work of Stefani⁶⁸ is the only data set that has examined the mechanisms behind the gender difference during performance in anaerobic events, several other studies have examined this difference in laboratory-based studies. These studies have typically reported that males tend to display higher absolute peak (35–72%) and mean (~40%) power during anaerobic tasks such as the vertical jump and the Margaria-Kalamen and Wingate test.^{65–67,69} Maud and Shultz⁶⁵ reported that males possessed a greater (45.6%) vertical jump height than females, though this difference decreased to 13% when corrected for total BM. No difference was observed in this data set for vertical jump height when both genders were corrected for LBM. Similarly, this trend was also observed across a maximal 5-s cycle sprint on a wind-braked ergometer.⁶⁵ These data suggest that body composition has a large impact on performance, in particular during explosive performance tasks.

The impact of muscle mass on longer-term anaerobic power and capacity is also demonstrated from several studies.^{1,63–69} A large gender difference has been reported in peak (45–72%) and mean (48%) anaerobic power across a Wingate test. However, this difference is substantially reduced when corrected for total BM (15–35%; 20–26%), LBM (2.5–19%; 7.5–17%), and active lower-body muscle mass (ALBMM) (8.3%; 9.9%), respectively.^{65,66,69} Interestingly, large differences between genders were reported for the absolute peak (121%) and mean (66%) power outputs across

a maximal arm cranking exercise.⁷⁰ However, these gender differences were also reduced when corrected for BM (72%; 33%), LBM (55%; 21%), and active upper-body muscle mass (AUBMM) (42%; 9%). Therefore, the data available demonstrate that correction of anaerobic power for total BM and LBM reduces the gender difference in particular during cycling, whereas a considerably large effect still exists for upper-body anaerobic exercise. This most likely reflects the difference in body composition and development of upper-body musculature that is more typical of males.

Mayhew and Salm⁶⁶ reported that absolute anaerobic power across the Wingate test was significantly correlated to height, BM, LBM, %BF, and absolute leg extension strength in a large male college cohort. The same correlations were observed with female anaerobic power, with the exception of %BF. Further, Mayhew and Salm⁶⁶ reported that in short explosive anaerobic tasks, LBM, strength, and %BF accounted for 83% of anaerobic performance in almost all cases. Further, Murphy et al.⁶⁷ reported that differences in thigh volume, BM, and LBM explain the majority of variation in mean (48%; 74%; 79%) and peak (53%; 71%; 76%) power. Interestingly, Weber et al.⁷⁰ demonstrated that the correlation between both peak and mean power outputs across a Wingate test increased in strength when corrected for total BM ($r = 0.95$; 0.95) and LBM ($r = 0.92$; 0.91) when compared to the ALBMM ($r = 0.83$; 0.86), respectively. Similar relationships were reported for these body-composition indices and the power output from the upper body across a maximal arm-cranking effort. In support of this finding, Batterham and Birch⁶³ have also reported that anaerobic performance is independent of volume of active musculature. This observation may suggest that other factors such as neuromuscular functions as well as differences in bioenergetics and hormonal responses may play a contributing role to differences observed in physical performance between genders.

4.3.2.2 Bioenergetics and Energy Metabolism

Energy provision during anaerobic performance is dependent upon several key biochemical pathways that occur within the muscle. The suggestion of a gender-based difference in anaerobic metabolism is difficult to identify, particularly as the majority of data suggests no difference in anaerobic performance when corrected for lean muscle mass. However, a number of researchers have suggested that the anaerobic capacity of females is between 70 and 85% of that of men.^{1,67,72–74}

Hill and Smith⁷³ observed a significant difference in the energy contribution from aerobic and anaerobic metabolism during a 30 s Wingate test. The researchers demonstrated that women completed only 50% of the work during the 30 s test, although when made relative to total body mass the difference between genders was reduced to 30%. When separated into the contributions of aerobic and anaerobic work, men produced higher absolute (34% and 55%) and relative to body mass (35% and 7%) values than women, respectively. However, when made relative to total work, women contributed a significantly higher relative percentage of aerobic work than men (25% vs. 20%). Moreover, data from Weber and Schneider⁶⁹ demonstrates that indices of anaerobic metabolism such as the maximal accumulated oxygen deficit (MAOD) are significantly different between genders (50%), even when corrected for BM (21%), LBM (12%), and active muscle mass (17%). This suggests that other physiological factors besides body composition are responsible for changes in anaerobic

metabolism between genders. Weber and Schneider⁶⁹ reported that this difference was not the result of an increased ability of males to produce lactic acid or to secrete catecholamines. It has also been demonstrated that during anaerobic performance, neither lactate production nor sympatho-adrenergic responses are altered across the separate menstrual cycle phases.⁷⁵ Weber and Schneider⁶⁹ suggested that the differences in muscle fiber distribution and associated enzyme activities between genders may be a contributing factor in this difference.

Equivocal findings have been presented in numerous data sets reporting on the gender-specific difference in muscle fiber distribution.^{5-7,30,76,77} It has been reported that women typically have smaller fiber cross-sectional areas, in particular of Type IIA and IIB/X.^{5,71,76,77} As such, a larger relative area of the muscle is composed of Type II fibers in males and this may possibly alter the metabolic capacity of the muscle.⁵ Although no difference appears to exist in muscle fiber distribution, the biochemical environment within the muscle appears to show some differences between genders. Jaworowski et al.⁵ reported that active men possessed significantly higher activities in anaerobic enzymes such as phosphofructokinase (PFK) (27.6%) and lactate dehydrogenase (LDH) (25.5%) when compared to women. This supports past research that there is a clear difference in anaerobic enzyme activity between men and women.^{6,30,77,78} These data have also reported that other anaerobic enzymes such as pyruvate kinase, calcium-stimulated ATPase, phosphorylase, and m-LDH are typically between 15 and 35% higher in men. As such, males tend to have higher anaerobic capacities than females, which may support the observed differences in performance.

Interestingly, Esbjornsson et al.⁷⁷ demonstrated that despite this, there was no direct gender effect observed in muscle metabolites across a Wingate test. The subsequent changes in energy metabolites did not differ between men and women in either Type I or II fibers following the 30 s sprint. Similarly, the Type II fibers demonstrated no gender difference in lactate concentration or glycogen reduction following the Wingate test. However, females demonstrated a significantly smaller (42%) reduction in glycogen and lower lactate concentration (20%) in Type I fibers following the test. This may be suggestive of a gender-based difference in carbohydrate metabolism during such high-intensity anaerobic exercise, which may be related to the gender-specific difference in 17- β -estradiol reported during exercise.⁷⁹ Therefore, from the available literature it appears that while there are a few gender differences in energy metabolism processes activated during anaerobic exercise, these are quite specific and do not appear to be of sufficient magnitude to individually influence performance.

In summary, the effect of body composition and gender on anaerobic performance is relatively apparent from the existing literature. The majority of published data suggests that anaerobic performance is largely related to body mass and, in particular, lean body mass. A small number of studies suggest that the gender difference in anaerobic capacity exists regardless of any correction for body mass. As such, men typically have larger muscle fibers and a superior glycolytic profile within the muscle for the provision of anaerobic energy supplies, though this appears to have little influence on energy provision and reaction product metabolism. These physiological differences reported between genders are most likely the reason for the large separation between males and females in elite anaerobic performance events.

4.4 GENDER AND ENDURANCE PERFORMANCE

4.4.1 GENDER DIFFERENCES IN ENDURANCE PERFORMANCE

Despite a suggestion in 1992 that females may one day outrun males in competitive ultradistance events,⁸⁰ elite males appear to run approximately 10–15% faster than elite females across all endurance running race distances from 1500 m to marathon, with the gender difference narrowing as the race distance increases.⁸¹ However, at distances between 100 km and 1000 km, the difference is even larger, with females 20–30% slower than males.⁸²

The effect of gender on the age-related decline in performance was examined in a cross-sectional study of swimming performance from the U.S. Masters Swimming Championships where a greater rate of decline in swim performance was observed in females than males across all swim events from 50 m to 1500 m.⁸³ In endurance running events, the decrease in performance is greater in women compared to men, possibly due to either biological or sociological differences.⁸⁴ These authors suggested that these gender differences may partly be explained by selection bias. That is, there are a smaller number of female runners in the older age groups. This suggestion was supported by research examining the running times, age, and gender of 415,000 run performances in the New York Marathon between 1983 and 1999.⁸⁵ They observed that female marathon participation showed a significantly greater percentage increase in all age groups compared to the males. They also observed that the number of masters' participants over the age of 50 years significantly increased at a greater rate than their younger counterparts and that the finishing times for the top 50 male and female finishers over the past two decades showed significantly greater improvement in the masters' age groups than the younger age groups, especially in the older female athletes.⁸⁵

As discussed in detail earlier in this chapter, females possess higher body fat levels than males, and males possess a greater muscle mass and total body mass. These gender differences explain performance differences in weight-bearing sports. However, in swimming events, the increased flotation and smaller body size of females may increase flotation and reduce drag and resistance compared to males. Moreover, females have greater peripheral body fat distribution, causing their legs and arms to float higher in the water than males⁸⁶ and increasing the economy of swimming.⁸⁷ This may explain the observation that the difference in English Channel swim records between males (6 h 57 min) and females (7 h 25 min) is only 6.3%, a lot lower than the 20% gender differences seen in running events of the same duration.⁸² Therefore, the data available demonstrate that males possess greater endurance potential than females over a range of sports and this would relate back to the physical and physiological differences reported to exist between genders.

4.4.2 FACTORS INFLUENCING ENDURANCE DEVELOPMENT

For the purposes of this discussion, the model for endurance performance proposed by Coyle⁸⁸ will be examined. Coyle⁸⁸ proposed that performance velocity in endurance events is dependent upon a number of physiological factors, including:

- Maximal O₂ consumption (VO_{2max})
- Maximal heart rate
- Stroke volume
- Lactate threshold
- Economy of movement
- Muscle fiber type, morphology, and capillarization
- Aerobic enzyme activity

Apart from these above factors, previous research suggests that both muscle mass⁸⁹ and blood volume^{90–92} may affect endurance performance in male and female athletes.

4.4.2.1 VO_{2max}

Until puberty, the VO_{2max} of boys and girls appears to be the same.¹³ Beyond puberty, the average female's VO_{2max} is only 70–75% of that of the average male. In elite adult athletes from a range of endurance sports, the VO_{2max} of females remains 10–30% below that of elite male athletes across all ages.^{93–95} In both young⁹⁶ and older^{97,98} endurance athletes, VO_{2max} is a strong predictor of running performance, with stronger correlations observed between endurance performance and VO_{2max} in populations heterogeneous for VO_{2max}.^{84,99}

Coyle⁸⁸ has identified VO_{2max} as a major contributor to endurance performance. According to the modified Fick equation, VO_{2max} is the product of maximal heart rate, maximal stroke volume, and maximal arteriovenous oxygen difference.¹³ Arteriovenous oxygen difference is further influenced by a variety of factors, including muscle mass, the capacity of the blood to transport and relinquish oxygen (blood volume, hemoglobin), and the capacity of the working tissues to take up and utilize oxygen (capillarization, muscle fiber type, aerobic enzyme activity).¹³ Specifically, within a normal population, women typically possess VO_{2max} scores 15–30% lower than male counterparts.¹⁰⁰ These differences are 30–60% larger when VO_{2max} is expressed in absolute units (L·min⁻¹)^{95,101} but is significantly reduced to approximately a 20% and 10% difference when expressed relative to body mass (mL·kg⁻¹·min⁻¹) or fat-free mass (mL·kgLBM⁻¹·min⁻¹), respectively.^{95,101} These data suggest that body composition plays a major role in explaining the differences in VO_{2max} and thus endurance capacities between males and females.

For elite marathon runners, Cheuvront and others⁸² presented a table (Table 4.2) that summarized the physiological differences between elite males and females for the various indices that affect VO_{2max}.

This table reviews the results from a number of studies that measured both the VO_{2max} and the indices of VO_{2max} in elite male and female marathon runners. The table suggests 26% (L·min⁻¹), 17% (mL·kg⁻¹·min⁻¹), and 10% (mL·kgLBM⁻¹·min⁻¹) greater values in male versus female elite marathon runners when VO_{2max} was expressed in absolute terms, relative to body mass or relative to lean body mass. Taken together, the research strongly suggests that a female's lower total and lean body mass and greater body fat stores are major determinants of gender differences in endurance performance.

Gender differences in the rate of age-related decline in VO_{2max} are commonly observed in endurance athletes.^{93,102} For example, Brown and others⁹³ reported

TABLE 4.2**Physiological Comparisons between Elite Men and Women Marathon Runners for Indices of VO_{2max}**

	TBM (kg)	BF (%)	LBM (kg)	HR_{max} (b/min)	SV_{max} (mL/beat)	Q_{max} (L/min)	A-VO_{2max} (mL/100 mL)	Hb (g/dL)	VO_{2max} (L/min)	VO_{2max} (mL/kg/min)	VO_{2max} (mL/kgLBM/min)
Males	61.7	6	58.0	185	157	29.1	15	15.0	4.36	70.7	75.2
Females	54.6	13	47.5	186	115	21.4	15	13.5	3.22	59.0	67.8
Diff	7.1	7	10.5	1	42	7.7	0	1.5	1.14	11.7	7.4
% Diff	11.5	53.8	18.1	0.5	26.8	26.5	0	10	26.1	16.5	9.8

Note: TBM = Total body mass; BF = Body fat; LBM = Lean body mass; HR_{max} = Maximum heart rate; SV_{max} = Maximum stroke volume; Q_{max} = Maximum cardiac output; A-VO_{2max} = Maximum arteriovenous oxygen difference; Hb = Hemoglobin.

Source: Cheuvront, S.N., Moffatt, R.J., and DeRuisseau, K.C., Body composition and gender differences in performance, in *Nutritional Assessment of Athletes*, Driskell, J.A. and Wolinsky, I., Eds., CRC Press, Boca Raton, FL, 2002, pp. 177–99.

declines of $0.65 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{yr}^{-1}$ and $0.39 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{yr}^{-1}$ in male (17–64 yr) and female (16–54 yr) high-performance cyclists, respectively. Fitzgerald and others¹⁰³ used meta-analysis to suggest that $\text{VO}_{2\text{max}}$ in aging females declines at different rates in sedentary ($0.35 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{yr}^{-1}$), active ($0.44 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{yr}^{-1}$), and endurance-trained ($0.62 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{yr}^{-1}$) subjects. Wilson and Tanaka¹⁰⁴ also used meta-analysis to suggest age-related declines in $\text{VO}_{2\text{max}}$ of 0.40, 0.39, and 0.46 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\cdot\text{yr}^{-1}$ in sedentary ($n = 6,231$), active ($n = 5,261$), and endurance-trained ($n = 1,961$) males. Thus, it appears that the $\text{VO}_{2\text{max}}$ of older endurance-trained males and females declines with age at a faster rate than similarly aged cohorts.

An expansion of blood volume is a common finding following endurance training.^{105,106} A 20–25% expansion of blood volume that accompanies endurance training provides advantages of greater body fluid to facilitate enhanced thermoregulatory ability through sweating, a larger vascular volume for greater cardiac filling and stroke volume, and thus enhanced cardiovascular stability during both exercise and changes in posture.^{105,106} High correlations have been observed between $\text{VO}_{2\text{max}}$ and blood volume in both young¹⁰⁵ and older male¹⁰⁷ and female⁹² endurance athletes or aging individuals with high $\text{VO}_{2\text{max}}$ values.⁹⁰

Research also suggests that muscle mass differences between the genders can explain differences in $\text{VO}_{2\text{max}}$ and endurance performance. This is not surprising given the long-known relationship between maximum aerobic power and the amount of total body lean mass.¹⁰⁸ A number of studies involving maximal arm cranking¹⁰⁹ and walking on a treadmill¹¹⁰ have not shown any gender differences when $\text{VO}_{2\text{max}}$ is expressed per unit of appendicular muscle mass. These findings suggest that gender differences in muscle mass account for gender differences in $\text{VO}_{2\text{max}}$.

Apart from the body-composition differences between the genders discussed above, research has also consistently shown that females have a lower $\text{VO}_{2\text{max}}$ compared to males as a result of other components of the oxygen transport and utilization systems.^{12,13} These include

- Smaller heart size
- Smaller left ventricular mass
- Lower plasma volume
- Lower stroke volume
- Lower cardiac output
- Lower arteriovenous oxygen difference
- Lower hemoglobin concentration
- Lower blood oxygen carrying capacity

Research has shown that, at least in younger adult individuals, both males and females respond to endurance training in the same way. In both genders, $\text{VO}_{2\text{max}}$ increases approximately 15–30% depending on endurance training depending on training frequency, duration, and most importantly, intensity.^{111,112} Maximum heart rate does not appear to change as a result of endurance training in either gender.¹¹³ However, major increases in maximal cardiac output occur as a result of a significant increase in maximal stroke volume that is secondary to increases in both end-diastolic volume and end-systolic volume. These changes are primarily the result of

a significant increase in blood volume and stronger myocardium surrounding the left ventricle, respectively.^{114,115}

Endurance training also results in similar changes at submaximal endurance exercise intensities in both males and females. In both genders, cardiac output at any submaximal exercise intensity does not change in either gender. However, endurance training results in an increased stroke volume and a lowered submaximal heart rate at the same absolute workload.¹¹⁴ In both genders, there are small but significant increases in arteriovenous oxygen difference at any exercise intensity.¹¹⁶ In both genders the metabolic and morphological changes that occur with endurance training include

- Increased maximal muscle blood flow¹¹⁷
- Increased muscle capillary density¹¹⁸
- Increased mitochondrial density^{119,120}
- Increased oxidative enzyme activity^{119,120}

Research has shown that significant improvements in endurance performance occur in males and females of all ages, depending on the initial fitness level, genetics, and specific training frequency, intensity, and duration.^{115,121} In both young and older adults of both genders, aerobic capacity increases approximately 15–30%. In both young males and females, this increase in $\text{VO}_{2\text{max}}$ induced by endurance training is partly explained by increases in cardiac output as a result of increases in stroke volume¹²² and numerous peripheral adaptations discussed earlier. In contrast, it appears that there may be gender differences in the adaptations to endurance training in older individuals. For older males, it appears a significant increase in stroke volume and thus cardiac output explains the majority of the $\text{VO}_{2\text{max}}$ increase with a smaller but significant increase in arteriovenous oxygen difference explaining a minority of the $\text{VO}_{2\text{max}}$ increase. In contrast, in older females it appears a highly significant increase in arteriovenous oxygen difference contributes all of the observed increase in $\text{VO}_{2\text{max}}$.^{123,124}

While $\text{VO}_{2\text{max}}$ is widely regarded by exercise and sport scientists as the single best indicator of an individual's cardiovascular endurance capacity,¹³ the percentage of this capacity that an athlete can sustain during aerobic performance has been consistently shown to better predict endurance performance ability.^{125–127} This level of intensity is often referred to as the lactate threshold.¹³

4.4.2.2 Lactate Threshold

Endurance performance is not only determined by an individual's $\text{VO}_{2\text{max}}$.⁸⁸ Lactate threshold (LT) has been shown to be a better predictor of endurance performance in athletes than $\text{VO}_{2\text{max}}$. LT is defined as the point at which blood lactate begins to accumulate substantially above resting concentrations during exercise of increasing intensity.¹³ Coyle⁸⁸ determined that lactate threshold is a major determining factor in endurance performance. In modeling endurance performance, research has consistently shown that both VO_2 at lactate threshold¹²⁶ and velocity at lactate threshold are better predictors of endurance running performance than $\text{VO}_{2\text{max}}$.^{125,127} In younger distance runners. A longitudinal study by Wiswell and others¹²⁸ observed that lactate threshold as $\%\text{VO}_{2\text{max}}$ did not differ between male and female endurance runners and

increased significantly with age in both groups. Thus, lactate threshold appears to be similar between similarly trained males and females when expressed relative to $\text{VO}_{2\text{max}}$, suggesting there are no gender differences in lactate threshold.

4.4.2.3 Economy

In modeling endurance performance, Coyle⁸⁸ identified economy as a major determining factor in endurance performance. Economy is defined as the oxygen cost to exercise at a given exercise intensity (velocity or $\%\text{VO}_{2\text{max}}$) and has been shown to be a stronger predictor of endurance performance than $\text{VO}_{2\text{max}}$ in a homogenous group of endurance athletes.^{129,130} Moreover, studies that have examined the oxygen cost of running at the same relative intensity have shown no gender differences in running economy in young athletes.¹³¹ More recently, a study examined mechanical efficiency in recreationally active males and females matched for $\%\text{VO}_2$ peak values at ventilatory threshold and observed similar economy during both arm cranking and leg cycling.¹³² Similarly, Evans and others⁹⁷ tested the hypothesis that declines in 10 km run performance in females were associated with decreases in $\text{VO}_{2\text{max}}$, lactate threshold, and running economy. In thirty-one highly trained female runners aged 23–56 years, they observed that both 10 km performance and age were significantly correlated with $\text{VO}_{2\text{max}}$ and running velocity and VO_2 at lactate threshold. However, both 10 km performance and age were not correlated with running economy in the highly trained and competitive female endurance runners.⁹⁷

In summary, it appears that males and females adapt similarly to similar endurance training loads and have similar relative lactate thresholds and exercise economies. Thus, the available research contrasting males and females of all ages suggests that endurance performance differences between the genders are primarily the result of differences in $\text{VO}_{2\text{max}}$ values. In turn, this gender difference in $\text{VO}_{2\text{max}}$ appears largely explained by body composition differences such as the larger muscle mass of males and the greater percent body fat of females.

4.4.2.4 Muscle Mass

A decreased muscle mass has often been suggested as a contributor to the age-related decline in $\text{VO}_{2\text{max}}$ in sedentary aging individuals.^{89,133} Fleg and Lakatta⁸⁹ measured 24-hour urinary creatinine excretion (an index of muscle mass) in 184 healthy volunteers aged 22–87 years from the Baltimore Longitudinal Study of Aging. They observed a significant positive correlation between $\text{VO}_{2\text{max}}$ and creatinine excretion in both men and women. $\text{VO}_{2\text{max}}$ showed a strong negative linear relationship with age in both men and women. When $\text{VO}_{2\text{max}}$ was normalized for creatinine excretion, the variance in the $\text{VO}_{2\text{max}}$ decline attributable to age declined from 60% to 14% in men and from 50 to 8% in women, suggesting that muscle mass may influence the age-related decline in $\text{VO}_{2\text{max}}$ observed with age in healthy adults.

In summary, it would appear that endurance training into older age may not reduce the age-related loss of muscle mass observed in a sedentary aging population, but the quantity and quality of muscle may be enhanced through maintenance of contractile tissue. Moreover, it appears that the age-related decrease in muscle mass in particular contributes to the age-related decrease in $\text{VO}_{2\text{max}}$ and thus endurance performance in male and female masters athletes.

4.4.2.5 Blood Volume

Females have a smaller heart volume, lower hemoglobin, and smaller blood volume than males.^{115,134} The smaller heart volume leads to a smaller left ventricular mass in females, which, when combined with a lower blood volume, reduces preload, thus leading to lower stroke volumes and cardiac output commonly observed in female athletes and untrained individuals.^{115,134}

4.4.2.6 The Role of Estrogen

Recent research has observed that during endurance exercise females oxidize more lipid and less carbohydrate and protein than males.^{135,136} This increased lipid oxidation is related to higher intramuscular lipid storage and usage as well as greater adipocyte lipolysis. Moreover, females exhibit lower glucose appearance and disappearance rates than males and some evidence of glycogen sparing during endurance exercise. Circulating estrogens have been implicated as the hormonal reason for these gender differences in substrate metabolism.^{136,137} Estrogen appears to upregulate lipoprotein lipase activity during the luteal phase of the menstrual cycle, the phase of the cycle that has been shown to enhance endurance performance.^{137,138}

Tarnopolsky⁷⁹ has suggested that the gender differences in substrate metabolism are partially due to the higher levels of 17- β -estradiol in females. This suggestion is reinforced by research that has shown that 17- β -estradiol administration in males leads to lower carbohydrate and protein oxidation and higher fat oxidation during endurance exercise.⁷⁹

4.5 SUMMARY AND CONCLUSION

The influence that gender and body composition have on the several domains of exercise performance comprises several difficult concepts due to the interaction between the two variables themselves. As such, while considerable differences exist between genders with regards to physical performance, the causal factors are difficult to strictly determine. The ultimate goal of understanding the complex mechanisms underlying variations in exercise performance for both genders is to allow the development of safe strategies for maximizing exercise performance. Primarily, the characterization of hormonal profiles in distinct menstrual cycle phases, their physiological outcomes, and their effects on exercise performance indicators are of vital importance to the success of these strategies. The changes in hormonal profiles between genders appear to have several secondary effects on muscle mass, enzyme activities, and cardiovascular function, among other factors. It is evident from this review that further research is needed to attempt to identify these causal mechanisms. Clarification on these issues may allow female athletes to adjust competition and training schedules to their menstrual cycle in a further effort to maximize performance.

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Section III

Physical Activity Needs Assessment of Athletes

5 Laboratory Methods for Determining Energy Expenditure of Athletes

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5.1 INTRODUCTION

Determining energy expenditure for athletes can have a positive impact on their health and their ability to train and compete. Knowing the energy demands of practice can help the athlete maintain a positive energy balance (energy demands vs. energy intake), which optimizes the ability of the athlete to train and to increase muscle mass. For example, inadequate energy intake reduces the ability of the athlete to sustain training at high intensities or to exercise for extended periods, and has the potential to increase his or her risk of injury. Inadequate intake of energy also

reduces the anabolic muscle-building process. From another perspective, measuring maximal energy capacity can provide the athlete with information regarding the effects of the training program on metabolic adaptations. Also, knowing at what exercise intensity the athlete starts to produce significant lactic acid can assist the athlete in identifying the proper intensity at which to train to obtain maximal metabolic benefits. This chapter focuses on the present state of knowledge regarding the methods of measurement of energy use and provides some insights into how these measurements can benefit the athlete.

Most athletes think of energy in terms of the requirements for their exercise programs, but energy is also needed to build tissues, digest food, and create enzymes and hormones. The human body is not very efficient at utilizing the energy it produces, and about 80–85% of the energy that is produced is ultimately converted to heat. Thus, metabolism and heat production are typically viewed from the same perspective. For decades, the basic unit of energy for humans in the English system has been the kilocalorie (kcal). This is the amount of heat required to increase 1 kilogram (kg) of water one degree Celsius. However, the S.I. unit (le Système international d'unités) of the kiloJoule (kJ) is becoming more acceptable.¹ Conversion between systems is simple, as 1 kcal is equal to 4.184 kJ. Although these two units are used in the literature, other units of measure have been used. The kilopond-meter (kpm), or kilogram-meter (kgm), is a unit of work used frequently on cycle ergometers.² In this case the kilopond, or kilogram, is defined as the magnitude of the force exerted on one kilogram of mass. It is determined by multiplying the kilograms of resistance times the number of revolutions per minute, times the distance traveled per revolution. Thus, someone pedaling at 60 rpm on a standard cycle ergometer with a resistance of 2 kg would be working at 720 kgm: $60 \text{ rpm} \times 6 \text{ m/revolution} \times 2 \text{ kg}$. Kgm can be converted to either kcal or kJ in a two-step process, first converting to mL O₂ and then to energy units. There are approximately 2 mL of oxygen per kpm and 5 kcal/liter of oxygen. Using the example above, exercising at 720 kpm would use approximately 1.42 L O₂/min. At approximately 5 kcal/L O₂, energy use would amount to 7.1 kcal/min or 30 kJ/min. Some researchers have also reported work in units of watts (W); however, the watt is a unit of power (J/sec) and should not be used in energy production equations.

Energy derived from the complete combustion of each macronutrient is different. Fats produce the most energy per gram (9.4 kcal or 39.3 kJ/g), protein produces about 5.65 kcal or 23.7 kJ/g, while carbohydrates yield only 4.3 kcal or 18 kJ/g.³ These values were determined using a bomb calorimeter. The bomb calorimeter is a strong steel cylinder, resistant to high pressures, with a highly insulated water bath surrounding it.⁴ The food substance is sealed in the cylinder containing high-pressure oxygen. The food is electronically ignited and the heat production is computed by measuring the increase in the water temperature in the water bath, taking into consideration the volume of water encircling the calorimeter. In contrast to the bomb calorimeter, human metabolism is not as efficient at assimilating and using these substrates for energy. Thus, for practical purposes the energy production of carbohydrates and proteins is about 4 kcal/g (17 kJ/g), while fats produce approximately 9 kcal/g (37 kJ/g).⁴

5.2 METHODS FOR THE MEASUREMENT OF METABOLIC RATE

Our knowledge of the measurement of metabolic rate and energy expenditure during physical activity has changed little since the mid-1990s.⁵⁻⁹ The energy output of humans is still measured by direct and indirect calorimetry.⁵⁻⁹ The direct calorimetry method measures heat production or air flow. Presently, these are the most accurate methods, but most individuals do not have access to the expensive, complicated facilities and equipment needed to use these methods.⁵ Indirect methods, which rely on the measurement of oxygen uptake, are less expensive, smaller, and more portable than direct methods. Studies have shown good agreement between direct and indirect calorimetry.^{5,7} However, the advantages of indirect calorimetry are considerable and the use of indirect calorimetry gained popularity in the early 1900s.⁹⁻¹¹ Although these indirect calorimetry systems are less expensive than direct calorimetry, they are still outside the limits of cost for most individuals and are usually found in clinics or laboratories.⁵ However, as technology advances, costs should decline and systems will be in more widespread use in fitness centers.

5.2.1 DIRECT CALORIMETRY

Direct calorimetry assesses heat production and typically requires a small room with heavily insulated walls.^{5,7,11} These units are larger versions of the bomb calorimeter, using the same basic science to measure the metabolic rate. The walls of the unit contain a series of finned pipes through which water is pumped at a constant rate. The heat generated by the subject is measured by the difference between the incoming and outgoing water temperatures measured to the 0.01°C, knowing the volume and rate of the water flow. Oxygen is continuously supplied and carbon dioxide is removed by chemical absorbent. Direct calorimeters come in several sizes and types, ranging from suit calorimeters, like those used by astronauts, to small chambers and even larger rooms. In place of a water temperature gradient, the use of incoming and outgoing air flow has been tried and has met with some success for resting measure.⁵ Since the response time is slow,⁵ its use in sports is very limited. Using direct calorimetry to measure metabolic rate takes considerable time, as it takes at least 20–30 minutes for the heat loss and heat production to equilibrate.⁷ Therefore, direct calorimetry appears to work best for measuring resting metabolic rate or energy use during prolonged steady-state activities. The methods will not work for measuring daily energy expenditure or for most sports or activities because of the confined nature of the chamber. Another form of direct calorimetry is the isothermal system, which has a faster response time.⁵ The isothermal system uses a chamber lined with insulating materials and a water jacket. In these systems the temperature gradient across the insulating layer is proportional to the nonevaporative (sweat) heat loss of the person in the chamber. The response time of these units appears to be moderate (~5 min) and the measurement error is small⁵; however, this system would not work for most sports. In summary, none of the direct calorimetry methods will work to obtain acute metabolic measures, such as the maximal capacity or anaerobic threshold, that assist with training programs for athletes.

5.2.2 INDIRECT CALORIMETRY

The underlying principle of indirect calorimetry is that energy production requires oxygen.⁵⁻⁹ Thus, if the oxygen uptake is measured, energy production can then be estimated, and through mathematical conversion, the results can be presented either in kilocalories or kiloJoules. The equipment to obtain oxygen uptake has been found to have an error as little as 1% compared to direct calorimetry.¹⁰ Indirect calorimetry appears to be the method of choice for measurements of short-term energy expenditure at rest or during activity.⁸ The method is based on four assumptions.⁷ First, the individual is not in a starvation state. Second, since the individual is not starving, protein makes up only a very small portion of the energy and can therefore be ignored. Third, the contribution of anaerobic metabolism to the energy production is quite small. Fourth, when using a combination of carbohydrates, fats, and proteins as a source of energy, approximately 4.82 kcal (20 kJ) of energy is liberated per liter of oxygen used.¹¹ For convenience, the 4.82 kcal/L O₂ has been rounded to 5 kcal or 21 kJ per liter of oxygen. In general, in a normally fed individual performing steady-state exercise, these assumptions are true. However, for short-term (< 5 min) or anaerobic, high-intensity exercise, these assumptions are not accurate and indirect calorimetry cannot be used to estimate energy expenditure unless recovery measurements are obtained. Recovery measurements are necessary because the anaerobic energy will eventually have to be replaced by aerobic means. These recovery measurements can last from 15 to 60 minutes or longer, depending upon the nature of the activity.

There are actually two general indirect calorimetry methods. One employs a closed circuit system while the other uses an open circuit system.⁵ Both appear to be equally valid; however, the open circuit system has proven to be more advantageous for activities involving movement.

Closed-circuit spirometry uses a spirometer, an airtight cylinder, filled with 100% oxygen. The system also contains a carbon dioxide absorbent such as soda lime, which is used to remove the CO₂ exhaled in each breath.³ The person simply breathes the oxygen from the spirometer. Since oxygen is absorbed by the body and any CO₂ produced is removed from the spirometer, the volume of gas in the spirometer is reduced. The difference between the initial and the final volumes of oxygen in the spirometer is the oxygen uptake. The oxygen uptake is then multiplied by 5 kcal/L of oxygen to obtain energy use. There are some problems inherent with this system.² First, the system must be airtight so volumes will not change inappropriately. Second, the temperature of the gas will affect the volume in the spirometer. A 1°C rise in temperature will cause a 0.34% increase in the volume of air.² Since expired air is at a higher temperature than inspired air, failure to correct for this temperature differential leads to an underestimation of metabolic rate. Third, the CO₂ absorbent must be adequate or the CO₂ production simply replaces the oxygen uptake, providing the same absolute volume and reducing the measured oxygen uptake; CO₂ is then recycled and rebreathed. The inadequate CO₂ absorbent increases the CO₂, drives up respiration rate, and reduces any exercise performance. Also, at high metabolic rates, the CO₂ absorbent may not be able to keep pace with the respiratory CO₂ output, once again reducing exercise performance. Fourth, since the equipment is a

closed circuit, the apparatus must have the capacity to hold a large volume of oxygen. For example, during exercise a person may utilize 2–3 liters of oxygen per minute. Thus, for a 20-min run, the apparatus must be able to contain at least 40–60 liters of oxygen. Finally, once the subject is on the apparatus, the person cannot come off the apparatus until the final measure has been made or room air will enter into the system and reduce the oxygen uptake. These limitations, plus the bulky size of the equipment, the need for a large volume of pure oxygen, and the close proximity the subject needs to be to the equipment, have limited the use of closed-circuit spirometry for exercise studies.

The open-circuit system has proven to be useful in measuring energy both at rest and during exercise. In this method, the person does not rebreathe his or her air. The person simply inspires ambient air and expires air through a system of tubes with one-way valves so that measurements can be made of the total volume of air and the expired proportions of oxygen and carbon dioxide.^{7–9} The difference between the inspired and expired oxygen is the oxygen uptake (VO_2).

There are basically three major types of open-circuit systems: (1) a bag system, (2) a computerized system, and (3) a portable system.¹¹ All three systems start with the subject breathing through a mask or breathing valve that allows ambient air to enter and directs the expired air through gas analyzers. All three types contain a meter to measure total air volume (ventilation meter, turbine, or pneumotach), an oxygen meter, and a carbon dioxide meter. The bag system collects the volume of expired air in a large meteorological balloons or a standard rubberized Douglas bag.^{5,9} The contents of the bag are measured for the overall air volume and the concentrations of oxygen and carbon dioxide. These values are then introduced into a formula to compute oxygen uptake. The computerized system takes the output from the three meters (expired oxygen, expired carbon dioxide, and ventilation) and computes the oxygen uptake.¹⁰ The modern computerized system has the advantage of using instruments with much faster response time so that oxygen uptake can be captured breath-by-breath. Modern technology and microprocessors have resulted in miniaturizing the computerized systems to the point that they weigh less than 1 kg and can be worn on the back or abdomen, thus giving the person freedom of movement. Some of these portable units contain telemetry systems that allow investigators to obtain breath-by-breath information on energy expenditures for many activities with the person's movements unimpeded. Since the systems are lightweight, they contribute very little (1–2%) to the total energy of adults, but the weight of the unit could add 5–10% to the energy expenditure of a 10–20 kg child.

All of the open circuit systems have the same underlying assumptions as with other indirect calorimetry systems: The individual is not in a starvation state, protein makes up only a very small portion of the energy and can therefore be ignored, and the contribution of anaerobic metabolism to the energy production is quite small. However, the open circuit systems do not generalize the energy production from a liter of oxygen (5 kcal/L O_2). Instead, the open circuit methods utilize the fact that energy produced by carbohydrates and fats have different oxygen requirements (VO_2) and carbon dioxide production (VCO_2) rates.¹² Using open circuit spirometry to measure energy expenditure requires that the exerciser attains steady state. This is because the VCO_2 and VO_2 only represent substrate utilization during steady state.

In this state the VCO_2 is usually less than the VO_2 , so the relationship, or respiratory exchange ratio (RER), is always ≤ 1.0 . The respiratory exchange ratio (RER), respiratory quotient (RQ), or simply the R value, is the ratio of VCO_2 to VO_2 uptake.⁷⁻⁹ The RER does not take protein metabolism for energy into consideration; therefore it is sometimes referred to as the nonprotein RER.^{7,9} This method of computing energy expenditure will not work for individuals in a starvation state in which they are utilizing considerable protein. The RER for carbohydrate is 1.0, as the oxidation of a single glucose molecule requires six oxygen molecules and produces six carbon dioxide molecules, or a ratio of 6:6 ($\text{CO}_2:\text{O}_2$), which equals 1.0. The chemical reaction is $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{energy}$.³ Conversely, the oxidation of fats requires more oxygen and produces less CO_2 . For example, the metabolism of a molecule of palmitic acid, a typical fatty acid used for energy, uses 23 oxygen molecules and produces 16 CO_2 molecules ($16/23 = 0.696$), summarized by the following chemical equation: $\text{C}_{16}\text{H}_{32}\text{O}_2 + 23\text{O}_2 \rightarrow 16\text{CO}_2 + 16\text{H}_2\text{O} + \text{energy}$.³ As the composition of the substrate used for energy changes from fat to glucose, the RER changes from 0.7 to 1.0. An individual consuming a 50:50 mixture of carbohydrates and fats has an RER of 0.85. In addition to revealing the source of energy, the RER also relates to the amount of kilocaloric production per liter of oxygen.^{12,13} Carbohydrates produce 5.047 kcal/liter of oxygen uptake (21 kJ/L), while fats only produce 4.686 kcal/liter of oxygen uptake (19.6 kJ/L).

At the onset of exercise or during any high-intensity activity, lactic acid will be produced from anaerobic metabolism. Lactic acid is buffered by bicarbonate ions in the blood, causing an increase in CO_2 output ($\text{H}^+ + \text{HCO}_3^- \rightarrow \text{H}_2\text{O} + \text{CO}_2$). The increased VCO_2 will rise above what is expected for aerobic metabolism, causing the RER to be > 1.0 and abolishing the ability to compute energy expenditure.⁷⁻⁹ This is the major limitation of indirect calorimetry.

The best method to compute energy expenditure from open circuit spirometry involves the respiratory exchange ratio, so the oxygen uptake and carbon dioxide production must be measured.^{5,7,9} To accomplish this, six factors must be known: Inspired volume of air (V_I) per minute, inspired percent of oxygen ($F_I\text{O}_2$) and carbon dioxide ($F_I\text{CO}_2$), the expired air volume (V_E) per minute, and the expired percentages of oxygen ($F_E\text{O}_2$) and carbon dioxide ($F_E\text{CO}_2$). The ventilation, either V_I or V_E , is measured directly from a gas meter, pneumotach, or turbine. Knowing either V_I or V_E , the other volume can be calculated using the Haldane conversion.⁹ The volume of air needs to be corrected, since barometric pressure, temperature, and relative humidity affect the volume and not the percentage of the gases.⁹ The correction allows for comparisons of oxygen uptake obtained in the desert or below sea level with those obtained in the high rain forests or on a cold mountain top. The standard correction factor is to adjust barometric pressure (P_B) to sea level (760 mmHg), temperature to 0°C , and relative humidity to 0%. This factor is known by the anachronism of *STPD*: standard temperature, pressure, and dry.⁹ Failure to apply the factor can result in a 7–15% error in the overall calculation of energy expenditure (see Table 5.1). The $F_I\text{O}_2$ and $F_I\text{CO}_2$ are known (20.93% and 0.03%, respectively), while the $F_E\text{O}_2$ and $F_E\text{CO}_2$ are obtained from monitoring expired air using O_2 and CO_2 meters. The computational formulas are presented in Table 5.1.

TABLE 5.1**Calculating Energy Expenditure from Measurements of Oxygen Uptake (VO_2) and Carbon Dioxide Production (VCO_2) and Respiratory Exchange Ratio (RER)**

$$V_{\text{STPD}} = [(P_B - \text{water vapor pressure})/760] \times [273/(273 + \text{Temp of the gas})]$$

$$V_E = V_I \times [0.794/(1 - F_E\text{O}_2 - F_E\text{CO}_2)]$$

$$\text{VO}_2 = (V_I \times F_I\text{O}_2) - (V_E \times F_E\text{O}_2)$$

$$\text{VCO}_2 = (V_E \times F_E\text{CO}_2) - (V_I \times F_I\text{CO}_2)$$

$$\text{RER} = \text{VCO}_2/\text{VO}_2$$

RER on chart will give kcal/L oxygen (percent carbohydrates and fats)

$$\text{kcal/min} = (\text{kcal/L O}_2) \times \text{VO}_2 (\text{L/min})$$

$$\text{kJ/min} = (\text{kcal/min}) \times 4.184 \text{ kJ/kcal}$$

Note: V_I and V_E refer to ventilation volumes of inspired (I) and expired (E) air; $F_I\text{O}_2$ and $F_E\text{O}_2$ refer to fractions of inspired (I) and expired (E) oxygen; and $F_I\text{CO}_2$ and $F_E\text{CO}_2$ refer to fractions of inspired (I) and expired (E) carbon dioxide.

Sources: Consolazio C.F., Johnson R.E., and Pecora L.J., *Physiological Measurements of Metabolic Functions in Man*, McGraw-Hill, New York, 1963, pp. 1–98; Krogh, A. and Lindhard, J., *Biochem. J.* 14, 290–363, 1920; McArdle, W.D., Katch, F.I., and Katch, V.L., *Exercise Physiology: Energy, Nutrition and Human Performance*, Lippincott Williams & Wilkins, Philadelphia, 2007; and Zuntz, N. and Schumburg, N.A.E.F., *Studien zu Einer Physiologie des Macsches*, A. Hirschwald, Berlin, 1901.

The oxygen uptake values, obtained from the formulas in Table 5.1, are expressed in units of liters of oxygen per minute (L/min). This is considered the absolute VO_2 . The oxygen uptake can also be expressed taking into consideration body mass: milliliters of O_2 per kilogram body weight per minute (mL/kg/min). This is considered to be the relative VO_2 . The absolute VO_2 is used to obtain overall energy expenditure. Individuals who have large amounts of muscle mass, are tall, or are considered obese usually have a larger absolute VO_2 than normal-sized individuals. When trying to compare the energy expenditure of individual of differing sizes, relative VO_2 may be the preferred unit of measure. However, larger or taller individuals (including obese persons) usually have lower relative aerobic power (expressed per kilogram body mass) because their weight has a greater contribution of bone, connective tissue, and/or fat than a smaller individual, and these tissues do not contribute to overall oxygen consumption in the same way that muscle mass does. To overcome these limitations, some scientists have suggested using the units of mL O_2 per kilogram fat-free mass (mL/kg_{FFM}/min). This is an area in need of further explorations.

In 1936, D.B. Dill¹⁴ proposed a system of expressing energy expenditure in increments of resting metabolic rate—thus the origin of the metabolic equivalent or the MET. The MET has been defined as 3.5 mL/kg/min, or 1 kcal/kg/hr,^{9,11,15} which is thought to represent the average resting value for an adult; however, this value is debatable.¹⁶ The MET has become a popular unit of measure in epidemiological studies of activity or clinical studies in which maximal graded exercise testing is an integral part. This is because most research has shown that the energy cost of weight-bearing activities (for example, walking or running) is fairly similar per kilogram

weight or per MET.^{11,14,15} Furthermore, tables have been developed for estimating energy expenditure of most physical activities, including work, home, transportation, and sports activities.¹⁷ In terms of METs, a normal individual has a maximal capacity of 10–13 MET, while highly trained endurance athletes can have a MET capacity of 20–24 METs.

5.2.3 DOUBLY-LABELED WATER

Thus far, the methods described for measuring metabolic rate have limitations. They have the potential for precision, but some methods restrict movements, while others are limited to gathering information during only minutes or hours of use. None of the methods relate well to non-steady-state activity or very high-intensity activity in which CO₂ output can become greater than oxygen uptake (anaerobic work). In an attempt to overcome these problems, a technique using doubly-labeled water has been developed.^{5,18–21} Doubly-labeled water is an isotope of water in which both the hydrogen and oxygen are tagged with a tracer, ²H₂¹⁸O. The underlying principles of the technique are that the hydrogen from the doubly-labeled water is eliminated as part of the water and the oxygen is eliminated both as part of the water and carbon dioxide molecule. Since there is equilibrium between the oxygen molecule in the water and the carbon dioxide, it is possible to measure the carbon dioxide production by measuring the hydrogen and oxygen isotope in the body's water.²¹ The energy expenditure is computed based on total body water, daily CO₂ output, and isotope turnover in the urine. The subject simply consumes a dose of the labeled water. The dose is based on estimated total body water. The subject then goes about his or her activities for a period of 5–7 days and collects his or her urine. The isotope turnover in the urine is measured by high-precision mass spectrometry. The overall error of this method is about 6–8%; however, considering this is over a week, the error is acceptable.^{5,21}

The doubly-labeled water method is based on six assumptions.^{19,21} First, the volume of water in which the ²H₂¹⁸O is diluted is constant. This is not quite true because eating and drinking behavior is episodic rather than constant and some individuals are losing or gaining weight. However, this difference turns out to be less than a 1–2% error.²¹ Second, the fluxes of water and CO₂ are constant. Although this is not true due to the episodic nature of physical activity, eating, and drinking, once again the difference appears to be not quantitatively important.²¹ Third, the body water compartments act as a single compartment with respect to the equilibration of isotopes. This assumption has proven to be controversial as the hydrogen has been noted to be more rapidly exchangeable than the oxygen. To avoid the equilibrium issue, some investigators have used dilution space correction factor rather than total body water. Fourth, the rate of tracer influx exactly represents the rate of tracer efflux. The analytical model has been adjusted for this. Fifth, no CO₂ or water enters the body through the skin or lungs. Because the aim is to measure the dietary water intake and CO₂ production, any additional environmental sources would cause an error. Cigarette smoking can increase CO₂ intake, thus inducing a 3–6% increase in the estimate of energy expenditure. Although some exchange occurs in a nonsmoking person, once again the error is quantitatively unimportant.²¹ Sixth, the food quotient, obtained from dietary intake, is used to estimate the dietary mix rather than the

respiratory exchange ratio. This is important since the heat production (energy) per unit of CO_2 differs by about 30% when comparing carbohydrates and fats. The use of the food quotient introduces approximately a 3% error. This assumption does not account for alcohol intake or if energy intake differs considerably from energy expenditure. The overall effect of the inaccuracies of these assumptions is to induce a 2–8% error depending upon dose and duration of the study.^{18,20}

The major problem of the technique is the expense of equipment necessary for the isotope and total body water analyses and the expense of the dosages of $^2\text{H}_2^{18}\text{O}$. However, other problems have been reported. Speakman et al.²² found that the error for estimating VCO_2 from doubly-labeled water was not normally distributed and could result in an error for duplicate samples of 3–47%. In defense, this error could be reduced considerably by analyzing the samples in sets of five rather than two. Roberts et al.²³ have reported that the quality of isotope (amounts of $^2\text{H}_2$ and ^{18}O) may differ, resulting in substantial variability, including some physiologically impossible results. Although these problems exist, $^2\text{H}_2^{18}\text{O}$ presently provides our best estimate of free-living energy expenditure. This method appears to work well for obtaining estimates of overall energy expenditure but will not work to obtain an athlete's maximal capacity or anaerobic threshold, both of which are commonly used in the development of training programs for athletes.

5.2.4 INDIRECT METHODS OF ESTIMATING ENERGY EXPENDITURE

Since metabolic equipment is costly, requires considerable training to use, and is difficult to use for normal activities of life, indirect methods have been used in an attempt to estimate energy expenditure (EE). These methods include the use of heart rate monitors, motion sensors, and even thermal imaging.⁵

The use of heart rate to estimate energy expenditure has been explored because it is a relatively inexpensive method that allows the individual to be assessed in a free-living state.²⁴ Heart rates have the potential to provide information on the pattern of activity as well as a general estimate of energy expenditure. The use of heart rate monitors to estimate energy expenditure requires planning and calibration, since individuals vary in both resting and maximal heart rate. Thus, the person must first undergo testing so that the resting and maximal heart rates are known, and a heart rate/energy expenditure relationship developed. This usually requires the use of an ergometer for exercise and the measurement of oxygen uptake to compute energy expenditure. The subject then wears a heart rate monitor for a 24-hour period. The heart rates are downloaded to a computer and then averaged in 1- to 15-minute time segments. The average energy expenditure during that short time segment is then estimated by using the previously determined EE/heart-rate relationship and multiplying that by the number of minutes of activity. This procedure is then used repeatedly and totaled until the entire 24-hour EE is obtained.

The major problem with this method is that not all heart rate increases are related to changes in metabolic rate.^{24,25} Emotional stress and temperature changes are known to affect heart rate independent of metabolic rate. Thus, heart rates below 120 are not considered usable to determine energy expenditure.²⁵ In addition, heart rate only represents metabolic rate when a steady-state of activity has occurred. Thus,

during anaerobic activities or activities that have a considerable isometric component in which heart rates are elevated above metabolic rate, the use of heart rate can skew the results. Finally, the heart rate may not be sufficiently sensitive to respond to short-term activities.^{24,25} Levine⁵ suggests that the error of estimating metabolic rate from heart rate during low-intensity exercise is between 3 and 20%. Therefore, it appears to be impractical to use heart rate to estimate metabolic rate. However, heart rate can be used to estimate minutes of moderate- to hard-intensity activities.²⁴ More commonly, heart rates are used by athlete to understand their minute-by-minute training intensities rather than to estimate energy expenditure.

Motion detectors have also been used to estimate energy expenditure. Motion detectors can be as simple as a pedometer or as complex as a three-dimensional or omnidirectional accelerometer.²⁶ Pedometers only measure ambulation and thus are of limited value when calculating energy expenditure.²⁷ Accelerometers contain a piezoresistive microswitch that responds to motion. The units are small and usually worn on the hip. Some of these are designed to respond to movement in a single plane of motion (up–down) while others have the capability to respond in three dimensions. These units usually require a computer interface. The investigator enters the subject's age, sex, height, and weight and resets the unit to zero counts (initializes the unit). The subject then wears the unit for a period of time, sometimes even up to 7 days. The motion counts are then downloaded to a computer. The accelerometer has a built-in energy expenditure prediction equation based on sex, age, height, and weight. Energy expenditure is then derived by multiplying the motion counts by a prediction equation. The accelerometer output can be divided up into segments as little as 1 sec., giving the ability to compute EE during a specific activity as well as overall EE.

In adults, the uniaxial accelerometers appear to slightly overpredict energy expenditure during those activities that involve ambulation, like level walking or running.^{15,28} However, they underpredict the energy cost of activities that involve arm movement or external work, like stair climbing or hill walking.^{29,30} In addition, the units are ineffective for measuring EE for activities that do not involve ambulation, such as swimming, cycling, weightlifting, or any seated activity. Triaxial or omnidirectional monitors appear to be slightly more accurate, but they still appear to have the same limitation as the uniaxial models with respect to evaluate intensity of activity, arm work, or nonambulatory activities (such as swimming, weight lifting, and cycling).^{26,30} In addition, the formulas to compute energy expenditure were derived from adult data, which does not directly apply to children.³¹ Thus, motion detectors have a limited ability to estimate energy expenditure. They will not work to obtain maximal capacity or anaerobic threshold used for training programs for athletes.

Estimating energy expenditure from thermal imaging has also been attempted.⁵ Thermal imaging estimates heat loss from the body to the environment. Early studies showed little promise because of the slow response time, but more modern, high-resolution scanning has improved the accuracy during steady-state exercise but not during acute, short-term (< 10 min) exercise.

5.3 ENERGY FOR WORK AND SPORT

The energy expenditure of daily life is greater than the resting energy expenditure (REE) and is dependent upon lifestyle and occupation. A typical sedentary adult (for example, an office worker) will expend 140% of the REE. A teacher’s lifestyle will expend 160% of the REE, a nurse’s lifestyle about 170% of REE, while a physical laborer (brick layer) will expend up to 200% times the REE.^{4,17,2,32} These calculations do not include any energy used for an exercise program. This energy expenditure of exercise training is in addition to the daily need (REE + Lifestyle). For an adult who exercises about 30–45 min a day, this will amount to 250–450 kcal (1000–1900 kJ), or only about 10–14% of the total caloric expenditure. However, for the athlete who exercises 3–5 hours a day, the energy demand of the exercise alone can be greater than the total energy of REE plus lifestyle. Table 5.2 summarizes the estimated additional energy needs of individuals training for specific sports.³³ These estimated energy needs should not be taken as absolutes, only as examples of additional caloric needs. Note that some sports, like recreational basketball, may require only slightly more than normal amounts of energy, while others, like the Tour de France cycle race can require an enormous amount of additional energy. The actual energy demand of the exercise is based on the intensity and duration of the exercise, the type of activity, and the sex and weight of the exerciser. The weight of the athlete is why the American football player requires more energy than the endurance cyclists. The energy demands of activities that are weight bearing will be directly related to the athlete’s weight, whereas activities that are non–weight bearing, like bicycling,

TABLE 5.2
Estimates of the Energy Requirements for Training of Various Sports

Sport	Additional Energy Needs		Average Daily Energy Intake	
	Kilocalories	Megajoules	Kilocalories	Megajoules
Basketball	300	1.26	2200	9.20
Crew	600–5000	2.51–20.92	2400–7000	10.04–29.29
Dancers	1000	4.18	1500	6.28
Gymnasts	1400	5.86	1400	5.86
Football players	2100	8.79	4000–5300	16.74–22.18
Lacrosse players	700	2.93	3000	12.55
Runners (men)	1000	4.18	4400	18.41
Runners (women)	500	2.09	2400	10.04
Swimmers	500	2.09	2900	12.13
Tour de France	4000	16.74	6700	28.03
Triathletes	1500–2000	6.28–8.37	4095	17.15
Wrestlers	200–1000	0.84–4.18	Varies with weight	
Weightlifters	1400–4600	5.86–19.25	3000–4700	7.17–19.66

Note: In most sports the energy requirements for women are about 15–20% less than for men.
Source: Short, S.H. and Short, W.R., Four-year study of university athletes’ dietary intake, *J. Am. Diet. Assoc.* 82, 632–45, 1983.

TABLE 5.3**A Comparison of the Energy Demands (kcal/min) of Running (a Weight-Bearing Activity) and Cycling (a Non-Weight-Bearing Activity)**

Body Weight	Running	Cycling
	~ 6 mph (9.6 km/h) kcal/min	~ 9 mph (14.5 km/h) kcal/min
110 lbs (50 Kg)	9.7	5.0
130 lbs (59 Kg)	11.4	5.9
150 lbs (68 Kg)	13.1	6.8
170 lbs (77 Kg)	14.9	7.7
190 lbs (86 Kg)	16.6	8.6

Sources: Swain, D.P., The influence of body mass in endurance bicycling, *Med. Sci. Sports Exerc.* 26, 58–63, 1994; Swain, D.P. and Leutholtz, B.C., *Metabolic Calculations Simplified*, Williams & Wilkins, Baltimore, MD, 1997, p. 14.

require less increments of energy as the individual's weight increases (Table 5.3).³⁴ Thus, an overweight or obese athlete who wants to increase energy expenditure is better off walking than riding a bicycle. Interestingly, this overweight athlete actually utilizes significantly more energy to walk at a given speed than a nonobese athlete, but riding a bicycle at the same speed the overweight athlete uses just slightly more energy than a lean athlete.

Energy expenditure during activity is usually measured by open circuit spirometry. As previously mentioned, at this point in time, a miniaturized, computerized system appears to work best. Some of these systems are stationary and will only work with activities in which the participant is within 5–6 ft of the measurement device. Such systems have been used to measure energy cost of walking and running on treadmills, cycling on cycle ergometers, swimming using a swimming ergometer, rowing using a rowing ergometer, stair stepping using an escalator-type or step ergometer, elliptical trainers, or arm ergometer. The treadmill allows the subject to walk or run at specific speeds while maintaining a stationary location. Although the treadmill simulates ambulation, it is not quite the same as normal walking or running as the person's legs are propelled backward off the treadmill rather than the leg propelling the body forward. Also, there is a difference in air resistance between treadmill and normal ambulation that may decrease the energy cost of ambulation on treadmill.^{35,36} The same is true for cycle ergometers, as they eliminate air resistance; however, the ergometer also eliminates the friction of the tires on the riding surface. With swimming and rowing ergometers, the problem is that they eliminate water resistance (drag forces, frontal resistance, and skin or surface friction).³⁷ Water resistance is considerable; therefore, the use of swimming ergometers may underestimate the true energy expenditure of swimming. To overcome some of the drag issues, swimming flumes have been developed. The flume is like the "endless pools" in that the water is forced past the swimmer and the swimmer swims against that force or current to stay in place.

The bag technique of open circuit spirometry has been used to measure oxygen uptake during ergometry work as well as during actual cycling, swimming, rope skipping, or household chores.^{5,6} Since the expired air bag is connected to the subject by a breathing tube, this technique requires the researcher to move with the subject yet not impede any of the subject's movements. The subject usually wears a mouthpiece and has to support the breathing tube during the collection period. The weight of the breathing tubing, breathing valve, and mouthpiece can be uncomfortable for the subject or cause additional effort to support the apparatus and maintain the mouthpiece in the mouth. The air collections bags are typically bulky and hard to control during exercise. They are not totally impermeable to gas exchange and if used for longer than 10–15 minutes, gases may diffuse and the results can be unreliable. Therefore, bag measurements are usually taken over periods of time lasting less than 10 minutes and the contents measured as quickly as possible at the end of the collection period. The bag method can be used successfully but takes preparation, training, and good timing to obtain accurate data.^{5,9,11}

The use of miniaturized portable systems has revolutionized our ability to obtain energy expenditure data during activities.⁶ These new systems are small enough to be worn during activity, providing little impairment of motion. The systems have been used to measure energy expenditure of household chores, basketball, inline skating, cycling, tennis, and kayaking, to name a few.³¹ Some of these systems include a fairly large memory or telemetry, which allows the investigator to obtain hours of real-time data without being tethered to the subject.

Although these systems have proven to be accurate, there are some minor problems. As mentioned previously, the additional weight of the apparatus can increase the energy cost of the activities. It is also important that the systems be securely attached to the subject. If not, the system can impede motion, which will modify the energy cost. Most of these systems require that the subject wear a mask to measure expired gases, rather than the cumbersome breathing valve and mouthpiece. An improperly fitting mask can result in air leaks that can modify both the measured volume of air and the fractions of expired gases. Experience has also shown that the systems may lose their ability to function via telemetry if they are near an electric field such as a video display. Proper consideration and planning can eliminate these problems and allow the investigator to obtain accurate data.

5.4 METABOLIC MEASUREMENTS SPECIFIC TO ATHLETES

5.4.1 AEROBIC POWER OR $\text{VO}_{2\text{MAX}}$

Two metabolic measures that athletes are generally interested in are the maximal metabolic rate, referred to as their $\text{VO}_{2\text{MAX}}$, and their lactate threshold. $\text{VO}_{2\text{MAX}}$ is the maximal amount of oxygen that can be consumed and is considered by many to be the most valid indicator of aerobic fitness. A high $\text{VO}_{2\text{MAX}}$ is particularly important for endurance athletes. For example, a 2-hour, 20-minute marathon run is performed at about 11.5 mph (18.5 kpm). To maintain that speed on a level surface, an oxygen uptake (VO_2) of about 65 mL/kg/min is required.³⁸ From Table 5.4, the normal $\text{VO}_{2\text{MAX}}$ for a sedentary adult is around 30–4 mL/kg/min; thus, the normal

TABLE 5.4**Aerobic Power or $\text{VO}_{2\text{max}}$ (mL/kg/min) for Different Athlete Groups Presented by Sex**

Sport	Females	Males
Basketball players	38–52	38–53
Cross-country skiers	55–65	58–85
Cyclists	53–70	56–80
Distance runners	48–71	55–81
Gymnasts	36–49	45–55
Ice hockey players	35–55	50–61
Kayakers	50–57	40–50
Rock climbers	50–55	No Data
Rowers	46–55	55–65
Sailors	50–64	45–55
Soccer players	43–56	55–68
Surfers	52–60	50–80
Swimmers	58–60	54–68
Sedentary adults	30–48	35–49
Speed skaters	46–55	56–81
Sprinters (< 400 m)	No Data	47–71
Tennis players	44–61	50–70
Triathletes	47–58	55–72
U.S. football players	No Data	32–56
Weight lifters	No Data	36–61
Wrestlers	No Data	54–65

Sources: Obtained from our laboratory and from McArdle, W.D., Katch, F.I., and Katch, V.L., *Exercise Physiology: Energy, Nutrition and Human Performance*, Lippincott Williams & Wilkins, Philadelphia, 2007; Wilmore, J.H. and Costill, D.L., *Training for Sport and Activity: The Physiological Basis of the Conditioning Process*, Wm. C. Brown Publishers, Dubuque, 1988; Draper, N. and Hodgson, C., *Adventure Sport Physiology*, Wiley-Blackwell, Chichester, U.K., 2008; Mendez-Vallanueva, A. and Bishop, D., Physiological aspects of surfboard riding performance, *Sports Med.* 35, 55–70, 2005; Pluim, B.M., Stall, J.B., Marks, B.L., Miller, S., and Wiley, D., Health benefits of tennis, *Br. J. Sports Med.* 41, 760–68, 2007; and MacDougall, D.J., Wenger, H.A., and Green, H.J., *Physiological Testing of the High-Performance Athlete*, Human Kinetics Books, Champaign, IL, 1991.

adult could not run at that speed because the required metabolic rate could not be attained (among other reasons).^{11,40–44} Although there is a genetic component to $\text{VO}_{2\text{max}}$,³⁹ hard training can result in as much as 30% improvement, but normally the improvements are about 10–15%.^{11,40} The $\text{VO}_{2\text{max}}$ of different types of athletes can be found in Table 5.4. They were obtained from a variety of sources and measurements obtained at the University of North Carolina. The values reported in Table 5.4 simply represent a wide range of responses and should not be considered for success in athletics. One reason for the wide range of values is body size. For example, if one

examines the $\text{VO}_{2\text{max}}$ of the U.S. football player, there are values that are lower than for some sedentary adults, yet we know these athletes can sustain more exercise than a sedentary adult.⁴⁵ A normal adult male is about 75 kg and 1.78 meters tall, while most football linemen weigh about 130 kg and are about 1.98 meters tall. Because of the larger muscle mass of the football player, he will usually have a higher absolute $\text{VO}_{2\text{max}}$ (L/min) than the smaller, sedentary person. From Table 5.4, and knowing the mass in the above example, an estimate of 2.1 L/min and 4.2 L/min can be calculated for the normal adult and the football player, respectively.⁴⁵ Consequently, the football player has a higher overall metabolic rate, but when expressed per kilogram body mass, the rate is lower. Thus, care should be taken when interpreting the $\text{VO}_{2\text{max}}$, and attention should be paid to the units in which it is expressed. Clearly, endurance athletes need high $\text{VO}_{2\text{max}}$, but success in many athletic endeavors does not hinge on aerobic power.

Maximal oxygen uptake is usually measured by a progressive, incremental exercise test on an ergometer.^{44,46–48} The test is also referred to as a graded exercise test (GXT).^{46,47} The test starts out with a warm-up and then progresses in a stepwise-fashion from a low workload (for example, speed, resistance, or rpm) to the maximal workload that can be sustained by the individual. Each workload (step) typically lasts from 1 to 3 minutes, with an optimal duration of 10–15 minutes for the whole test. The test termination is usually subjective and considerable motivation is needed to have the individual attain true maximal capacity. However, there are some criteria validating that the person attained $\text{VO}_{2\text{max}}$: failure to keep up with the exercise protocol, plateau or a slight decline in VO_2 with increasing workload, reaching maximal heart rate and failure to rise with increasing workload, and $\text{RER} \geq 1.10$, or lactate levels of 8 mmol/L or greater.⁴⁹

Maximal aerobic power can also be estimated from submaximal exercise testing.^{47,48,50} These tests usually have the person exercise on a calibrated ergometer and complete two to four stages of a maximal test or continue until their heart rates reach ~160 beats per minute. The heart rates or oxygen uptakes are measured the final minute of each stage. The assumption is that there is a predictable, linear relationship between steady-state heart rate and steady-state VO_2 , so using that heart rate or VO_2 data, maximal capacity is then predicted using a graph or an equation.

The heart rate and VO_2 (or workload) data are usually plotted against each other, with heart rate on the x axis and VO_2 (or workload) on the y axis. An example is presented in Figure 5.1. A straight line of “best fit” is drawn and then extended out to the individual’s predicted maximal heart rate. (The maximal heart rate is predicted from $220 - \text{age}$.) A line is then extended to the y axis to obtain the VO_2 at that maximal heart rate, with that VO_2 value representing the $\text{VO}_{2\text{max}}$. Alternatively, the relationship between the heart rates and oxygen uptakes can be calculated mathematically (computing slope and intercept of the line) and $\text{VO}_{2\text{max}}$ can be determined from that equation.

These tests vary in accuracy, with correlations of 0.6 to 0.85 (moderate to strong) between measured $\text{VO}_{2\text{max}}$ and submaximal predictions.⁴⁸ The benefits of this testing are: (1) the results do not rely on the person pushing himself or herself to maximal limits, (2) they take less staffing, (3) they are safer with less physical risk, (4) require less time to run and interpret, and (5) many can be completed using a cycle ergometer or treadmill and a heart rate monitor, making them less expensive.⁴⁸ On the other hand, there are limitations especially for trained endurance athletes. Factors

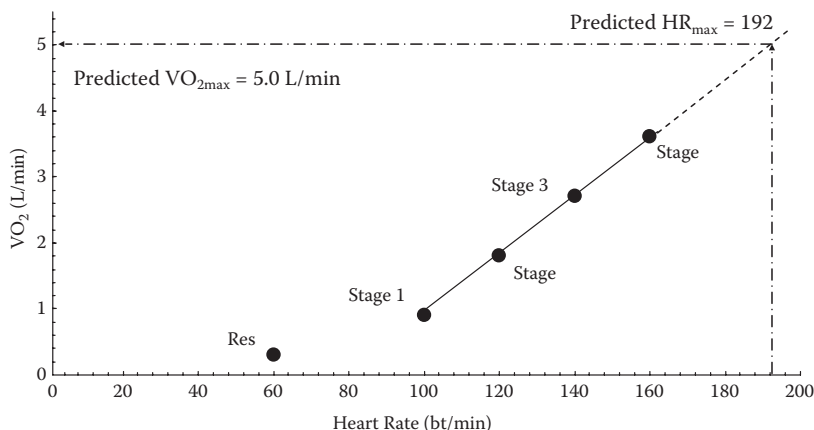


FIGURE 5.1 Predicting $\text{VO}_{2\text{max}}$ from heart rate and oxygen uptake for an individual who completes four stages of an exercise test with heart rate and VO_2 measured. The individual has a resting heart rate of 60 beats/minute and a predicted maximal heart rate of 192 beats/minute (on a cycle ergometer 300 kpm = 0.9 L/min VO_2 ; thereafter, each increment of 300 kpm = 0.6 L/min VO_2).

like dehydration, prior exercise, and environmental conditions (temperature and humidity) and length of test can influence the heart rate and therefore the accuracy of the results.⁴⁸ Athletes typically take longer than nonathletes to achieve the work rates normally needed to produce results. The longer the test, the greater chance that the athlete will elevate his or her core temperature, which will drive up heart rate over and above what would normally occur for a given work rate. The higher heart rate will result in an underprediction of maximal capacity. Most of the standard submaximal tests rely on heart rate and the prediction of age-appropriate maximal heart rate (HR_{max}); typically $\text{HR}_{\text{max}} = 220 - \text{age}$.^{47,48} However, many trained athletes have lower than predicted maximal heart rates. Thus using the $220 - \text{age}$ prediction equation could result in an overprediction of maximal capacity. In addition the anaerobic threshold cannot be determined from this form of testing as it can from the true $\text{VO}_{2\text{max}}$ test. Thus, as noted above, the accuracy of the predicted value is not that good. Submaximal tests can be used for athletes as a test–retest to determine if the exercise training program is progressing as expected. However, a simpler way to check progress is to have the athlete complete a standard exercise test and simply look at performance or heart rate. For example, a runner could do a time trial for a specific distance and then simply compare times. A swimmer could do a standardized set of interval training (sets of 100 to 200 m) and look at average times or heart rates. This would accomplish as much as the submaximal test. In summary, there appears to be limited value in using specific submaximal tests for athletic populations.

5.4.2 ANAEROBIC THRESHOLD

The anaerobic threshold is another metabolic measure that has several applications for athletes.⁴⁴ There are several terms commonly used: the anaerobic threshold (AT),

the lactate threshold, or the onset of blood lactate (OBLA).⁴⁴ Regardless of the terminology, the anaerobic threshold is the level of exertion at which there is a considerable increase in the use of anaerobic energy systems, causing the production of lactic acid.^{44,46} Lactic acid (referred to as lactate) is associated with fatigue, and the higher the lactate levels the shorter duration an athlete can exercise. For example, at a lactate level of 2 mmol/L, an athlete can usually exercise for more than an hour. At 5 mmol/L, the athlete may only be able to exercise for 20–30 minutes, and at 8 mmol/L the athlete may only last 2–5 min.^{11,40,50} The application of this is that when two athletes with the same $\text{VO}_{2\text{max}}$ are running at the same pace, the one producing less lactate will be able to sustain the run for a longer period of time with less fatigue. Also, the athlete with the higher AT will be able to run faster without the detrimental effects of lactate. This is why the AT of most marathon runners is typically > 80% of $\text{VO}_{2\text{max}}$, whereas the threshold of sprinters is typically in the 55–65% $\text{VO}_{2\text{max}}$ range.⁵⁰ This is clearly an advantage for most endurance athletes.

The anaerobic threshold can provide an estimate of endurance potential when expressed as a percentage of $\text{VO}_{2\text{max}}$. Athletes with a high proportion of fast-twitch fibers have the potential to produce considerable lactic acid at lower intensities of exercise. So if a coach is trying to determine if an athlete would make a better sprinter than a long-distance runner, one way would be to measure the athlete's $\text{VO}_{2\text{max}}$ and AT. If the AT is at a low proportion of $\text{VO}_{2\text{max}}$ (~60%) and the $\text{VO}_{2\text{max}}$ is not very high (50–55 mL/kg/min), then the athlete *might* be better at sprint events. If the AT is low and the athlete has a high $\text{VO}_{2\text{max}}$ (> 55 mL/kg/min) then the athlete *could* be better at middle distance events or may have the potential through training to be a good endurance athlete. If the athlete has a fairly high $\text{VO}_{2\text{max}}$ and the AT is a high percentage of that maximal capacity, then the athlete could be better at endurance events. Of course this method is not foolproof. Finally, at some point the endurance athlete can reach his or her aerobic potential and $\text{VO}_{2\text{max}}$ cannot be changed significantly with training. However, training can increase the anaerobic threshold.^{40,50} If the threshold increases as a proportion of $\text{VO}_{2\text{max}}$, then the athlete can exercise at a higher intensity with less buildup of fatigue-related lactate. Finally, the anaerobic threshold can be used by coaches and athletes during training to optimize the exercise intensity for maximal training results.^{40,44,50}

The anaerobic threshold can be measured during an incremental $\text{VO}_{2\text{max}}$ test.^{44,46} Usually the stages last about 3 minutes and increments in workload are smaller than for a normal $\text{VO}_{2\text{max}}$ test. Oxygen uptake and heart rates are obtained the last minute of each stage and blood lactate is sampled at the end of each stage. For runners, cyclists, cross-country skiers, skaters, or rowers, a sport-specific stationary ergometer can be used for the test. The test can also occur in a field setting, in which the athlete exercises at specific incremental speeds in 5-minute stages and blood lactate levels are measured between stages. This approach has been commonly used with swimmers.

Two common lactic acid demarcations have been used to determine the threshold: a lactate level of 2 mmol/L or 4 mmol/L.⁴⁴ For athletes, the 4 mmol/L threshold appears to have more relevance.^{40,44,50} The concept is to find the exercise intensity at which the threshold level of lactate is reached. The exercise intensity could be related to speed (for swimmers and runners), heart rate (for any training athlete), or a

proportion of $\text{VO}_{2\text{max}}$. Training below the threshold optimizes the aerobic energy system and maximizes the duration of the training session. Training above the threshold improves the athlete's speed, power, and ability to buffer the fatigue-related acids but reduces the duration of the training session.

Measuring the anaerobic threshold using lactate requires blood samples and a machine to analyze the compound. Although small portable machines for measuring lactate are available, some individuals do not like to have blood sampled. To avoid the blood sampling and still obtain an estimate of the AT, some scientists have suggested the use of the relationship between ventilation and metabolic rate.⁴⁶ The theory is that in an aerobic state there is a consistent relationship between ventilation and metabolic rate (V_E/VO_2). However, when lactate is produced and buffered, the hydrogen ions and additional CO_2 cause an increase in ventilation, over and above what is required for metabolism. Thus, this substantial increase in ventilation is a “surrogate” for the anaerobic threshold. For example, the V_E/VO_2 ratio is normally about 25 L air/1 L VO_2 , but for some athletes it can be as low as 15/1. So the scientist simply computes the V_E/VO_2 ratio for each stage of the $\text{VO}_{2\text{max}}$ test and where an abrupt increase in the ratio occurs, that is the AT. Since some of the lactate is buffered and may not influence the ventilation, some researchers have suggested that both the V_E/VO_2 and V_E/VCO_2 ratios need to be taken into consideration.⁴⁶ With this method there must be a rise in the V_E/VO_2 without an increase in the V_E/VCO_2 . This method is controversial and probably represents only an estimate of the AT in normal, healthy individuals. Regardless of the methodology, the anaerobic threshold is a very useful metabolic measure for aerobic athletes.

5.4.3 ECONOMY OF MOVEMENT

As the distance of a competitive event increases, another metabolic measure comes into play: economy of movement. Economy is basically the energy expenditure per unit speed.⁹ Better economy of movement relates to sustaining endurance performance and delaying fatigue. Measurements of economy are typically used for sports like marathon and ultramarathon running, long-distance cycling, rowing, and paddling or swimming—events that drain the muscle and liver glycogen supplies. Fatigue in short events lasting 5 minutes or less is not related to depletion of muscle glycogen; however, in prolonged events glycogen depletion is central to fatigue.^{11,40} So a sprinter can “waste” some energy to attain the goal, whereas a long-distance cyclist must be as efficient as possible with metabolic resources to attain his or her goal. Consistent with this, a study of elite long-distance (> 10 km) and middle-distance (800 m) runners found that the long-distance runners used 5–11% less oxygen at a given speed.^{51,52} For long-distance swimmers (≥ 1500 m), technique has a very important role. Measuring economy as the swimmer trains can be an indicator of improvement in technique. The concept can also be applied to rower and paddlers.

Fortunately, measuring economy is not difficult. The athlete simply exercises at several speeds, attains a steady state at each speed, and the metabolic rate (VO_2) is measured. The ratio of VO_2 to speed is calculated. Since there is no “perfect ratio,” the characteristic is individually applied and measured several times during a train-

ing season to substantiate that training is producing the desired results.⁵³ Specifically, economy has improved when energy cost per unit speed has decreased.

A number of individual characteristics can influence economy. Fiber composition is one of those characteristics.⁵³ Three types of muscle fibers dominate skeletal muscle: slow-twitch oxidative (Type I), fast-twitch glycolytic-oxidative (Type IIa), and fast-twitch glycolytic (Type IIb). Slow-twitch fibers are the most mechanically efficient and the most aerobically energy efficient but do not produce large power outputs.⁵⁴ They can use both fats and glycogen for energy production.^{11,40,50} Fast-twitch glycolytic fibers produce the most power but are the least aerobically efficient and rely on glycogen as their primary source of energy. The fast-twitch oxidative-glycolytic fibers are a mix of the two extremes and end up to be the most “trainable” of the fibers, taking on the characteristics of training regimen (sprint or endurance). These differences in muscle characteristics are why successful long-distance athletes typically have more slow-twitch fibers, while successful sprinters have a high proportion of fast-twitch fibers. The fiber type distribution is also the reason that most sprinters cannot run long distances, and long-distance athletes are not successful at sprints.^{40,50} Of course there are exceptions!

Another factor contributing to economy is the physical characteristics of the athlete. Body size appears to be important for swimming, running, or cycling. Taller individuals have longer arms and legs, which appears to be an advantage. Shorter individuals require higher stroke frequencies at the same speed than a taller individual, and typically increased frequencies cause greater energy expenditure and less economy. The combination of size and muscle fiber type distribution may be contributing to the fact the women are more efficient at slower speed running but less efficient at high speeds.⁵⁵ However, body composition (greater fat content) and biomechanical factors (leg alignment) are also contributing factors to sex differences in economy.^{54,55}

Finally, equipment can have an impact on economy. In long-distance running, the weight of the shoes can have an impact on energy use. In swimming, the advent of the “laser” swimsuit (Speedo) reduces drag forces, improves economy, but is now against international rules. There are several examples in cycling as well, including bike and helmet designs that reduce drag forces and toe-clips that increase efficient use of energy throughout the pedal revolution.⁵⁶ Thus physical characteristics, technique, physiology, biomechanics, and equipment all contribute to economy of movement.

5.5 RESTING ENERGY EXPENDITURE

The resting energy expenditure (REE) accounts for about two thirds of the daily energy expenditure and is therefore important when analyzing an athlete’s energy balance. Resting energy expenditure is sometimes confused with the basal energy expenditure (BEE, or sometimes referred to as the BMR). The basal energy expenditure is the minimal amount of energy necessary to sustain life—the energy needed to keep the heart beating, respiration going, and maintain cell metabolism, nerve transmission, body temperature, and so forth. The BMR requires that the person have no additional physiologic or psychologic stimulation, such as digestion, excess

temperature regulation, psychological tension, or any form of physical activity or movement.^{56,57} It is usually measured with the person resting supine after at least 8 hours of sleep, and at least 12 hours after the last meal or exercise.^{6,57} On the other hand the resting energy expenditure (REE), or resting metabolic rate (RMR), is the energy expenditure required to maintain normal body functions at rest.^{8,11} The REE is typically measured in the morning after a normal night's sleep, with the individual lying down or sitting in a thermo-neutral environment, after a 12-hour fast, and not having exercised for 12 hours. Since the two states are relatively close in definition, and since the difference between the BMR and the REE is less than 10%, the terms are often used interchangeably.^{9,11} In fact, Schutz and Jéquier suggest that if the REE is measured in a postabsorptive condition, it is the same as BMR.⁸ However, they are really two differing states. True BMR is difficult to precisely measure and requires more controls than the REE and as a result, the REE is usually obtained. Both the BMR and the REE are usually expressed in kilocalories per hour (kcal/h) or kilojoules per hour (kJ/h). The rate varies as much as $\pm 20\%$ from individual to individual.^{8,11} Although REE provides basic information on resting energy expenditure, it does not provide information that could influence an athlete's training program. It might provide insights into the nutritional balance of the athlete when the athlete's performance is declining for no apparent reason.

5.5.1 MEASUREMENT OF RESTING ENERGY EXPENDITURE

Any of the methods of calorimetry can be used to measure REE. However, the REE is usually obtained from two 5- to 7-minute continuous measures of VO_2 and VCO_2 . In some cases a single 15-minute collection period is used, with the first 5 minutes of measurement discarded and last 10 minutes of measurement averaged to obtain the REE.^{6,58} The subject usually reclines in a supine position for approximately 30–45 minutes in a quiet, thermo-neutral environment, sometimes covered with a light blanket. The mask or mouthpiece is put into place so that the subject is breathing through the apparatus during this initial rest period. This reduces any anxiety caused by the equipment. The subject is told to remain fairly still but not to sleep. At the end of the initial rest period the measurements are made. The methods for measuring BMR need to be more restrictive to reduce subject awareness and anxiety and usually involve gas measurements obtained with the subject inside a transparent hood or using a room calorimeter.^{8,58} In addition the BMR measures are usually obtained over a 20- to 45-minute period rather than the two 5- to 7-minute measurements.^{7,58}

5.5.2 ESTIMATING RESTING ENERGY EXPENDITURE

Resting energy expenditure can be directly measured. The measurement takes considerable equipment, time, and knowledge; thus methods have been derived to estimate REE based on indirect measures of weight, height, and age. The simplest method is based on gender. Adult males will use 1.0 kcal/kg/h or 4.186 kJ/kg/h, while females will use 0.9 kcal/kg/h or 3.77 kJ/kg/h.^{4,6,8,59} The person's body mass (kilograms) is multiplied by the appropriate gender factor to obtain kcal/h. A variation on this simple method is to multiply the weight in pounds times 10. These

TABLE 5.5
The World Health Organization Equations for Estimating Daily Resting Energy (kcal/day) Expenditure Based on Age, Gender, and Body Mass

Age Range (yr)	Equation	
	Males	Females
0–3	$(60.9 \times \text{kg}) - 54$	$(61.0 \times \text{kg}) - 51$
3–10	$(22.7 \times \text{kg}) + 495$	$(22.5 \times \text{kg}) + 499$
10–18	$(17.5 \times \text{kg}) + 651$	$(12.2 \times \text{kg}) + 746$
18–30	$(15.3 \times \text{kg}) + 679$	$(14.7 \times \text{kg}) + 496$
30–60	$(11.6 \times \text{kg}) + 879$	$(8.7 \times \text{kg}) + 829$
> 60	$(13.5 \times \text{kg}) + 487$	$(10.5 \times \text{kg}) + 596$

Note: kg = body mass in kilograms
Source: World Health Organization, *Energy and Protein Requirements*, Report of the Joint FAO/WHO/UNU Expert Consultation, Technical Report Series #724, WHO, Geneva, 1985, p. 206.

methods are crude and do not take into consideration age, size, muscle, or fat mass; therefore, they should only be used to estimate REE. Since REE declines with age, the World Health Organization (WHO) improved upon these simple prediction equations by developing six age-within-gender prediction equations.⁵⁹ The WHO equations correlate from 0.60 to 0.97 with reported direct measurements of REE (Table 5.5). Table 5.6 summarizes and compares the results obtained by using the four different analytical methods to estimate REE. As evident from Table 5.6, there is over a 15% difference between methods of estimation, and there is no simple way to determine which formula is most accurate for which person. Generally, equations based on gender, age, weight, and height may be more accurate, usually within 10–15% of direct measures.⁴ These formulas, however, do not take into consideration extremes in muscle or fat mass. Thus, for the athlete who has larger muscle mass and less fat mass than a normal individual, the best means for obtaining REE appears to be some method of direct measurement.

5.5.3 FACTORS INFLUENCING RESTING ENERGY EXPENDITURE

It is important to note that not all calories ingested are usable. The processes of digestion and absorption, as well as assimilation of substrate in the liver (proteins, glycogen), after feeding require energy. These processes are about 65–95% efficient, dependent upon the type of food.^{3,8,58} Therefore, at rest 5–30% of the calories are given off in the form of heat.^{8,60} These heat calories are referred to as dietary-induced thermogenesis or specific dynamic action (SDA). The dietary-induced thermogenesis varies by substrate. Carbohydrates increase REE about 4–5%, while fats only increase REE by about 2%.^{3,8} Conversely, protein increases REE by 20–30% and ethanol is about 22%.^{3,8} A typical mixed meal would increase REE by about 5–10%. Dietary-induced thermogenesis usually peaks about an hour after eating and, if the meal is high in protein, the thermogenesis can last

TABLE 5.6
Examples of Computations Comparing Methods of Estimating Energy Requirements

Male: 20 years old	Female: 20 years old	
Height = 5'10" (1.78 m)	Height 5'5" (1.65 m)	
Weight = 163 pounds (72 kg)	Weight = 136 pounds (62 kg)	
1. REE = 10 × wtlbs	REE = 10 × wtlbs	
10 × 163 = 1630 kcal/d	10 × 132 = 1360 kcal/d	
2. REE = 1.0 kcal/kg/h	REE = 0.9 kcal/kg/h	
1 × 72 × 24 = 1728 kcal/d	0.9 × 62 × 24 = 1339 kcal/d	
3. WHO Equations Based on Age and Gender		
(15.3 × kg) + 679	(14.7 × kg) + 496	
15.3 × 72 + 679 = 1781 kcal/d	14.7 × 62 + 496 = 1407 kcal/d	
4. Equations Based on Gender, Weight (wt = kg), Height (ht = cm), and Age (a = yr)		
66.5 + (13.8 × wt) + (5 × ht) – (6.8 × a)	655 + (9.6 × wt) + (1.7 × ht) – (4.7 × a)	
66.5 + (13.8 × 72) + (5 × 179) – (6.8 × 20)	655 + (9.6 × 62) + (1.7 × 165) – (4.7 × 20)	
66.5 + 994 + 895 – 136 = 1819.5 kcal/d	655 + 595 + 281 – 94 = 1437 kcal/d	
Comparison of the Results of the Four Methods		
Method	Male Example	Female Example
1	1630 kcal/d	1360 kcal/d
2	1728 kcal/d	1339 kcal/d
3	1781 kcal/d	1407 kcal/d
4	1820 kcal/d	1437 kcal/d

for a considerable amount of time, 3 to 5 hours.⁸ The thermogenesis seems to be more dependent upon the feeding pattern than the total caloric intake, as feeding four meals produces a larger increase in thermogenesis than feeding one meal of the same caloric content.⁶⁰ However, gorging significantly elevates the thermogenesis,^{61,62} but the effect may not be as significant for obese individuals.⁶³ This is thought to be in some way related to their body fat.⁶³ Endurance training, on the other hand, may also lower the dietary-induced thermogenesis compared to untrained subjects.^{64–67} The reduced thermogenesis could help conserve energy during periods of intense physical training. Other factors that may influence dietary-induced thermogenesis include genetics, caffeine, nicotine, and diseases like diabetes mellitus that effect insulin.^{8,59}

The resting energy expenditure is directly influenced by the amount of metabolically active tissue, or lean body mass.⁸ The National Research Council reports that lean body mass accounts for about 80% of the variance in measuring REE.⁶⁸ Failure to account for lean body mass can produce erroneous results. For example, publications have reported that the 24-hour energy expenditure of highly active subjects was greater than sedentary controls.⁶⁹ However, when the expenditure was reported based on lean body mass, the groups were found to be similar.

Although the lean body mass has a major influence on REE, the size of the individual will modify that relationship. Size is concerned with the height for a given weight.⁶⁸ Nutritionists define size using body mass index, a weight–height ratio ($\text{wt}_{\text{kg}}/\text{ht}_{\text{m}}^2$), whereas physiologists use the body surface area to mass ratio (A_{D}/kg). Regardless of the units, the taller, thinner person will have a higher REE than the shorter, heavier person of the same weight. This difference is related to the fact that the taller, thinner person has more surface area through which heat is lost. Thus, the tall, lean person must produce more heat to maintain thermo-balance.

Age and gender are also significant factors affecting REE (Figure 5.2).⁷⁰ The total resting energy expenditure of children is less than adults, generally $< 75 \text{ kcal/h}$ (314 kJ/h) vs. $> 90 \text{ kcal/h}$ (377 kJ/h).⁶⁸ However, when expressed per unit of body weight, the expenditure of children is more than double that of an adult: 100 kcal/kg (418 kJ/kg) vs. $30\text{--}37 \text{ kcal/kg}$ ($126\text{--}155 \text{ kJ/kg}$).⁶⁸ The greater REE is related to growth and activity patterns.⁷¹ Once growth has stopped, the REE declines about 2% per year, relating to only about 100–150 calories in 50 years.^{68,70,72} Interestingly, lean body mass declines at a rate of about 2–3% per decade.⁶⁸ Thus, if the decline in lean body mass could be avoided, the age-related reduction in REE probably would not occur.

Figure 5.2 illustrates that there is little difference in the REE of boys and girls until about the age of 10 years. At approximately this time, pubescence starts and the body composition of the genders begins to differentiate. The boys continue to gain muscle mass, while the girls develop a greater proportion of body fat. This difference amounts to about a 10% greater REE in adult males. Thus, in general,

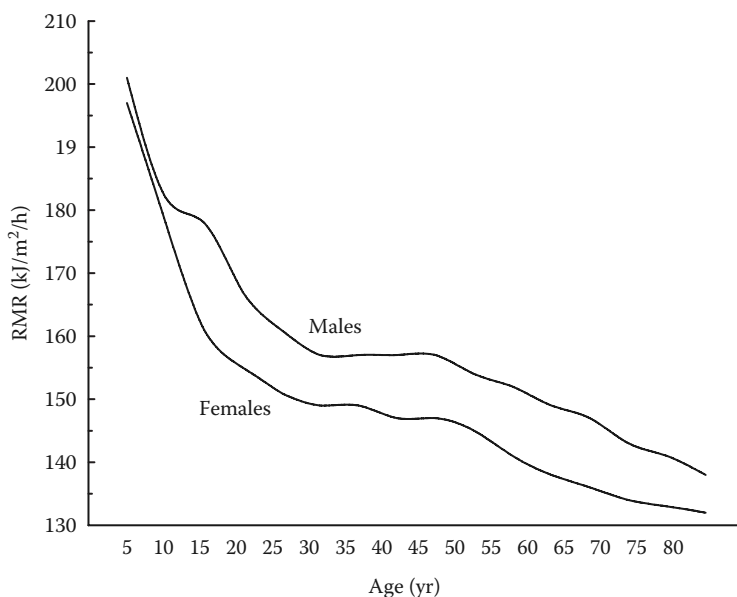


FIGURE 5.2 Relationship of age and gender to basal metabolic rate. The data is corrected for body surface area. (From Altman, P.L. and Dittmer, D.S., *Metabolism*, Federation of American Societies for Experimental Biology, Bethesda, MD, 1968.)

women expend about 0.9 kcal/kg body weight/hour. In contrast, men expend about 1 kcal/kg/h. This difference may not be a true gender difference but related to the greater body fat of females, or, conversely, to the greater muscle mass of males. This is verified by the fact that when metabolic rate is expressed per unit of fat-free mass (lean body weight), the comparative resting metabolic rates between men and women are very similar.^{16,70,73}

Other factors like climate,^{3,11,74} pattern of food intake,^{6,61,75} and hormones^{3,8,11,72} affect REE. Finally, exercise training may have an effect on REE (Figure 5.3). The effect of training on REE is controversial. Some studies have suggested that highly trained athletes have a greater REE per unit lean body mass than sedentary controls,^{64,70,76,77} while others disagree.^{66,67,78,79} The disagreement may be related to differing methodologies that have not controlled for a carryover effect of the previous exercise, which can persist up to 12–13 hours after prolonged strenuous exercise of 3–5 hours, the thermic effect of subsequent food intake, or the use of cross-sectional samples. Cross-sectional evidence suggests that highly active males and world-

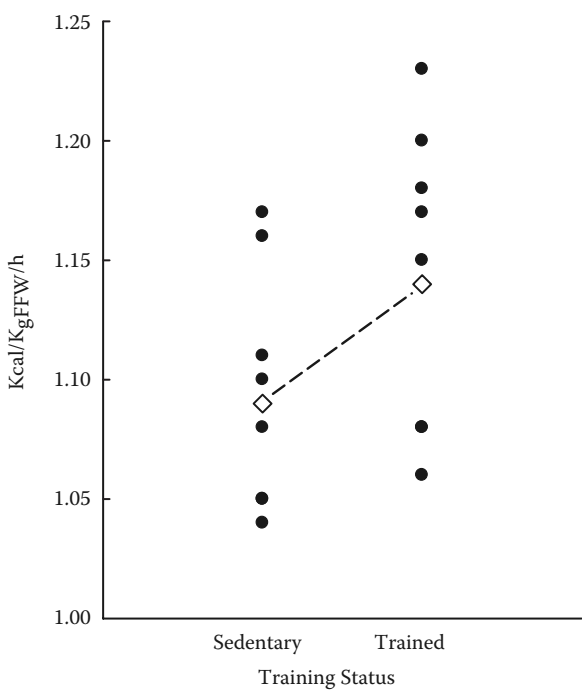


FIGURE 5.3 Effect of exercise training on the basal metabolic rate. The data presented with respect to lean body mass is a compilation of a number of studies. The open diamond (◊) represents the mean response for all studies in that group. (Data from Poehlman, E.T. et al., *Am. J. Clin. Nutr.* 47, 793–98, 1988; Davis, J.R. et al., *Eur. J. Appl. Physiol.* 50, 319–29, 1983; Poehlman, E.T. et al., *Metabol.* 41, 915–19, 1992; Tremblay, A. et al., *Int. J. Obes.* 10, 511–17, 1986; Wilmore, J.H. et al., *Am. J. Clin. Nutr.* 68, 66–71, 1998; Dolezal, B.A. and Pottleiger, J.A., *J. Appl. Physiol.* 85, 695–700, 1998; Poehlman, E.T. et al., *Brit. J. Clin. Pract.* 41, 684–88, 1987.)

class endurance athletes have a higher REE per unit lean body mass compared to moderately trained individuals.⁸⁰ At the other end of the spectrum, exercise training programs in sedentary individuals cause an elevation in RMR.^{77,81} Thus, there is accumulating longitudinal data to support an increased RMR with aerobic training. Also, the trained individuals usually have more lean body mass at a given weight, thus increasing absolute REE.

5.6 ESTIMATING DAILY ENERGY EXPENDITURE

The total, daily energy expenditure can be estimated by summing the REE, the daily activity factor, and the exercise program. For example, consider a 20-year-old woman who weighs 132 pounds (60 kg), works as a receptionist, and takes a 45-minute aerobics class 5 days a week. Her REE would be 1296 kcal per day ($60 \text{ kg} \times 0.9 \text{ kcal/kg/h} \times 24 \text{ h}$). Her daily activity would be an additional 390 kcal ($\text{REE} \times 30\%$). The aerobic program would expend about 275 kcals ($6.1 \text{ kcal/min} \times 45 \text{ min}$). Thus, on the days she exercises, her total energy expenditure amounts to 1961 kcal ($1296 + 390 + 275$) or 8.2 MJ/day, while on her nonexercising days she expends 1686 kcal ($1296 + 390$) or 7.0 MJ/day. By estimating the energy requirements for the day, the coach or a nutritionist can then combine this information with caloric intake to obtain caloric balance. A negative caloric balance (output greater than intake) of 3 days or longer can have an impact on the athlete's ability to train or perform, especially for endurance athletes. Conversely, a positive balance (intake slightly greater than output) allows the athlete to optimize his or her training and, in the long run, perhaps competitive performance.

5.7 FUTURE RESEARCH CONCERNS

Advancements in the measurement of energy expenditure allow us to obtain real-time data on energy expenditure during many different sports and activities for which we presently have only estimates. Researchers, scientists, and coaches now need to follow up and make these measurements for a variety of sports. However, these miniature metabolic systems are expensive and the risk for damage to this equipment or of injury is high for sports in which there is contact. So the use of these metabolic systems in many athletic situations is tenuous. In addition, having athletes wear the equipment during competition is not possible in many cases. So the best that can probably be accomplished is to measure the energy expenditure during practice and simulated situations. We have tried this for the sport of fencing.⁸² The fencer's mask was modified to accommodate a breathing mask from a miniaturized metabolic system, the system was moved from the front of the body to the lower back, and the back of the fencer was eliminated as a target for scoring. These modifications allowed for most foil, épée, and saber "touches" (scores) and also minimized risk to the equipment. Since fencing is ballistic in nature and metabolic rate could not be measured for a typical 10–15 second "touch" and the equipment slightly changes the fencer's normal competitive movements, we could only simulate a competitive bout and had to extend the

“sparring” for several minutes to obtain energy data. Attempts like this need to occur with other sports.

Another major issue is the measurement of anaerobic energy. Most sports are not performed at a constant rate or speed, so measurements of energy that rely on oxygen uptake are not directly applicable. As mentioned with the fencing example, most points are scored within 10–15 seconds of the start and this time frame is even faster for saber events. Most team sports, such as soccer, field hockey, or American football, have times when the athletes are sprinting for short period of time interspersed with rest or periods of light exercise. At present we do not have a means of accurately estimating the energy costs of these types of activities. Oxygen uptake will not work because these types of activities do not directly rely on oxygen. Measuring oxygen uptake and the exercise and recovery is one way to estimate the energy cost, but oxygen uptake during recovery is not only dependent upon the anaerobic demands of the exercise but also on any elevation in core temperature. Hence, during activities like fencing, where the athlete is wearing a uniform that reduces heat loss and consequently heats up, total energy cost of the sport would be overestimated. Heart rate may not relate to metabolic rate, so what is needed is a means of measuring anaerobic energy expenditure. A promising approach would be to use accelerometry in conjunction with prior titrating of energy expenditure (oxygen uptake) and accelerometer counts. This approach needs to be refined for specific athletes and specific sports.

One final issue in need of resolution is the effect of exercise training on resting metabolic rate. Such studies should be longitudinal in nature and should begin using unconditioned individuals and progress through the entire competitive season. The problem with this approach is that high-performing athletes usually do not become “unconditioned.” One approach may be to use athletes who are injured and cannot exercise for quite some time and follow their responses as they become conditioned. Resting metabolic rate studies also need to be completed on strength training athletes. Regardless of the group of athletes, measurements of metabolic rate need to be adjusted for fat-free mass (lean body mass) or changes in lean body mass that are a result of the training or detraining.

5.8 CONCLUSIONS

Knowing energy demands of an athlete and the metabolic responses to specific sports can provide an advantage to an athlete by improving nutritional balance and his or her ability to optimize exercise training and performance. However, the measurement of energy expenditure is a complex process that can be completed by several methods. Early studies employed direct calorimetry in which the person was placed in a closed chamber and the person’s heat production was directly measured. Since direct calorimetry confines the movement of the subject, there is limited application to athletes and physical activities. These limitations have led to the development of indirect calorimetry methods. These methods are based on the fact that the production of heat requires the use of oxygen and the production of carbon dioxide. Therefore, measuring the oxygen uptake and carbon dioxide production allows for the computation of energy use. Indirect calorimetry has evolved to the point where systems are sufficiently small so that subjects can exercise unimpeded, and metabolic

measurements can occur. Indirect calorimetry has a limited capacity to obtain data and has therefore been used mostly for measurements during short periods of time (such as minutes, hours). However, indirect calorimetry can provide the athlete with measures of both maximal capacity and anaerobic threshold—two characteristics that can be used as markers for training and that indicate the training regimen is working. Since indirect methods are not appropriate to obtain a measure of energy expenditure over a period of days, a double-isotope method using $^2\text{H}_2^{18}\text{O}$ has been developed. This method is most applicable when measuring overall (total) energy expenditure over days; however, it will not work to measure the specific energy cost of a given activity. Thus, it appears that indirect calorimetry is best for measuring specific activities, while the doubly-labeled water is best to estimate overall, daily energy use.

The resting energy expenditure (REE) can be defined as the minimal amount of energy necessary to sustain the human organism in a conscious, resting state. The REE makes up about two thirds of daily energy expenditure for a normal adult. In general, the REE is dependent upon the amount of metabolically active tissue, lean body mass. However, other factors such as age, gender, body size, climate, caloric intake, hormones, and exercise training will modify the REE. Resting energy expenditure can be measured by a variety of means ranging from room calorimeters that use direct calorimetry to simply measuring oxygen uptake, which is indirect calorimetry. Measuring VO_2 using a mask, hood, or even a whole room is the simplest means for obtaining an estimate of the energy expenditure. On the other hand, the measurement of energy expenditure during activity can be either quite simple or very complex depending upon the movements of the activity. Presently the best methods are through the use of portable, indirect calorimetry units. However, the measurement of energy expenditure from oxygen uptake requires that the activity can be completed in an aerobic state, which means that the activity is of a low-to-moderate intensity. At present, we have a limited capability to measure energy cost of very high-intensity exercise, which results in the production of considerable lactic acid.

Ultimately, to compute the individual daily energy expenditure, three factors must be summed: (1) the REE for the 24-hour period, (2) the energy expenditure based on lifestyle (work/school), and (3) the energy expenditure from any exercise program. For a sedentary adult, the first two factors (REE and lifestyle) coalesce to cause the vast majority of energy consumption. However, for some athletes their training programs can result in energy demands greater than the REE and lifestyle combined. For these athletes, knowledge of their energy needs can be vital for success in their sport.

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6 Field Assessment of Physical Activity and Energy Expenditure among Athletes

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6.1 INTRODUCTION

A large number of physical activity measures are currently utilized in the field setting to profile activity in terms of amount and type of movement performed, and many of these tools are also employed to predict energy expenditure. Physical activity is a multi-faceted construct, and although there are numerous techniques for assessment there is no “gold standard” measurement method.¹ In contrast, energy expenditure, which is a biological variable reflecting the sum of internal heat produced by external work, can be measured using well-accepted gold-standard methods. It is

important to remember that physical activity is a behavior that results in an elevation of energy expenditure above resting levels, and although these terms are often used interchangeably, they are inherently different and can be measured differently.² The techniques available to researchers, coaches, and athletes to measure physical activity and energy expenditure are vast, but the applicability of the different measurement tools will depend on the aspect of physical activity or energy expenditure that needs to be measured.

Historically, coaches have relied on personal experience to prescribe training programs to achieve optimal athletic performance; the trend now is to adopt a scientific approach to the development and monitoring of training programs.³ Enabling the more scientific approach in recent years has been access to the significant increase in commercially available devices to monitor and assess physical activity. The increased number of devices now available can be attributed to multiple factors, including the translation of laboratory-based approaches to the field, largely due to significant advances in technology and the production of lightweight portable devices. Consequently, the objective measurement of physical activity and quantification of energy expenditure in athletic and free-living contexts, across all age groups, is now possible using a wide range of measures. In choosing among the methods available, there commonly is a tradeoff between cost, participant burden, and the ability to assess specific features of the physical activity with ease of measurement, number of participants to be assessed, and time frame over which the assessment is to be made also considered.^{2,4,5} Given the strengths and limitations inherent in each of the methodologies available, there is value in using combined approaches in many situations. For example, objective measurement of daily total energy expenditure (TEE) and activity energy expenditure (AEE) of athletes using the doubly-labeled water (DLW) technique can be combined with heart rate (HR) monitoring and accelerometry, or global positioning systems (GPS) in some situations, to assess the number of bouts of activity and the duration and intensity of each bout. Furthermore, use of self-report instruments such as activity diaries provides valuable contextual information and subjective ratings of perceived exertion for the specific types or sessions of physical activity undertaken. Subjective diaries can also provide information regarding the psychological state of the athlete during and after training sessions or competitions.

To effectively manage the demands of athletic performance in training and competition requires a coupling between energy intake and expenditure to maintain a stable body weight. This necessitates a sound working knowledge of both food and energy, including the energy cost of physical activity. The assessment of physical activity and energy expenditure presents numerous challenges, including the affordability of techniques to the researcher or practitioner and the data collection burden on the athlete. As physical activity is a complex and multidimensional behavior, precise quantification is particularly challenging under free-living conditions.⁶ Numerous factors should be considered in the selection of assessment methods, including the age of participants, sample size, participant burden, method/delivery mode, assessment time frame, the type of physical activity information required, data management, measurement error, cost of the instrument, and others.^{4,5,7,8} It is important to appreciate that no single technique is able to quantify all aspects

of physical activity under free-living conditions; therefore, multiple measurement approaches are often used.

This chapter provides definitions of the major terms of importance followed by an overview of selected objective and subjective approaches used in athletic populations to assess physical activity and energy expenditure.

6.2 DEFINITIONS

The assessment of physical activity and energy expenditure has been affected by the inconsistent use of terms; therefore it is critical to provide an overview of key terminology. Physical activity is a global term and traditionally defined as bodily movement resulting from contraction of skeletal muscle leading to a substantial increase in energy expenditure above resting levels. In turn, physical activity can be categorized according to context or setting, for example, leisure-time or recreational physical activity, including sport, transportation, and occupational activity (Figure 6.1). In contrast, exercise is commonly defined as planned, structured, and repetitive movement with the intention of promoting or maintaining one or more components of physical fitness.⁹ The measurement of physical activity and exercise can therefore be complex given the variety of conditions in which an athlete lives, trains, and competes. Dimensions of physical activity and exercise include intensity, duration, frequency, and mode or type, for example, walking, running, swimming, or cycling. The duration of the activity or exercise refers to the time spent in the task, and frequency refers to how often one exercises, trains, or competes. Readers are referred to the paper by Howley,¹⁰ which provides a good overview of the various terms associated with physical activity and exercise, and provides guidelines for consistent interpretation of exercise intensity and volume.

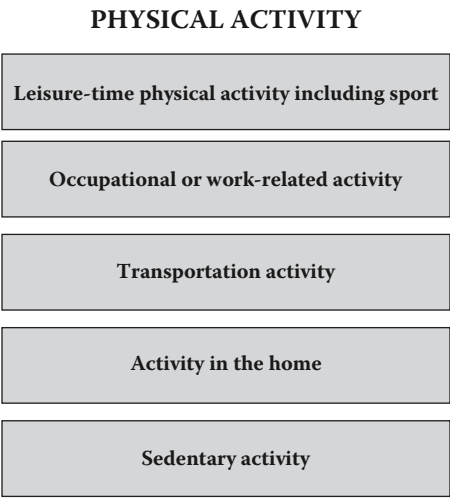


FIGURE 6.1 Components of total physical activity.

It is important to recognize that an outcome of participation in physical activity or exercise is the expenditure of energy that is commonly quantified in terms of intensity of effort. Intensity can be referenced in a number of different ways.¹⁰ For resistance weight training the training intensity is described as the relative weight lifted (%1-Repetition Maximum), and in aerobic exercise this is typically according to the elevation of HR in beats per minute. In other words, training intensity is the energy expended over and above the body’s basal metabolic requirements, and we can define physical activity broadly as activity energy expenditure (AEE) or more specifically as exercise energy expenditure (ExEE). AEE and ExEE can be expressed relative to resting values where 1 MET (metabolic equivalent) at rest has been considered to equate to 3.5 mL/O₂/kg/min⁻¹ or 1 kcal/kg/hr. It is important to note that the conversion of METs to kcal can be erroneous when using these standard conversion factors.^{11–15} However, we have shown that a correction factor based on measured or predicted resting metabolic rate (RMR) can reduce this error in some activities.¹²

Gross energy expenditure is quantified according to oxygen consumption and referenced in kcal/min or kJ/min to be more relevant to the individual, relative to body weight. In many athletic events and particularly in team sports, an overall assessment of the intensity of the event or game can be difficult given the often intermittent and variable pace and differences across playing positions. Figure 6.2 subdivides TEE into component parts and reflects the commonly used field assessment techniques to quantify these components.

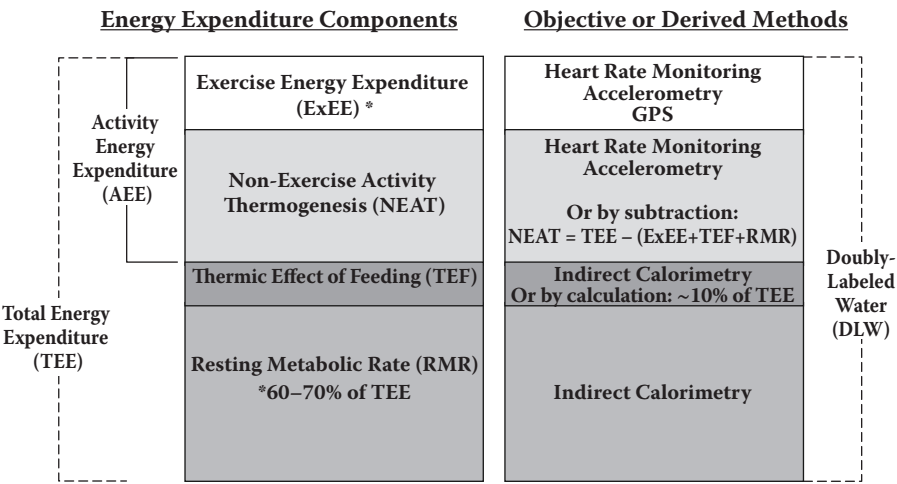


FIGURE 6.2 Assessment of TEE and techniques commonly used to quantify the separate components. It is important to note that each of the components listed in the diagram can also be measured directly using a whole-body calorimeter. However, the restrictive nature of the measure (participants are confined to a small room for the length of the measurement) means that free-living measurements are not possible. *ExEE and thus AEE are the most variable components; therefore, the proportions of TEE that RMR, TEF, and AEE represent differ between individuals.

6.3 METHODS OF ASSESSING PHYSICAL ACTIVITY AND ENERGY EXPENDITURE

6.3.1 OBJECTIVE MEASURES

Typically, more objective methods of assessing physical activity involve the systematic measurement of physiological and/or biomechanical parameters and the subsequent use of this information in profiling the amount and nature of the activity as well as in the determination of AEE.

6.3.1.1 The Doubly-Labeled Water (DLW) Technique

The DLW technique is the gold-standard or criterion measure to assess TEE.^{16–18} The major advantage of the DLW technique is that it is noninvasive and imposes minimal participant burden, which enables the assessment of TEE in athletes under normal living and training conditions over a 7- to 14-day period (depending on the analysis approach and age of the athlete). Another significant advantage of the technique is its accuracy and precision; however, the DLW technique provides a gross measure of energy expenditure and is not able to provide information on the nature or types of activity an individual has been engaged in.⁷ A further disadvantage is that although the sample collection can be undertaken in the field with little equipment or imposition to the participant, the sample analysis requires laboratory-based assessment using specialized equipment. Therefore the technique is prohibitive for large-scale, population-based assessments of energy expenditure. However, because of its gold-standard status, the DLW technique is commonly used to validate other techniques that are employed to quantify free-living energy expenditure, including physical activity questionnaires.^{5,19–21}

The DLW technique requires the collection of daily urine samples over 10–14 days, the measurement of RMR via indirect calorimetry (or prediction using equations), and specialist laboratory equipment and personnel for analysis.¹⁶ The two stable isotopes, deuterium (²H) and oxygen-18 (¹⁸O) are administered orally via a drink of water, and the elimination of the isotopes from the body is tracked by analyzing daily urine samples.^{22–24} The difference between the elimination rates of the two isotopes is equivalent to the rate of carbon dioxide production, which can then be converted to average daily energy expenditure.²⁵ The DLW technique provides an indication of daily energy expenditure across the measurement time frame. For a detailed overview of the DLW technique for the assessment of TEE, readers are referred to a recent publication by the International Atomic Energy Agency (IAEA).¹⁶

If RMR is accounted for, AEE can be calculated by assuming a constant for the thermic effect of feeding (TEF) (10% TEE):

$$\text{AEE (kcal/d)} = 0.9 \times \text{TEE (kcal/d)} - \text{RMR (kcal/d)}$$

AEE is therefore all the energy expended above resting and food ingestion–related energy costs. The ratio of TEE to RMR is a common measure of physical activity level (PAL); however, there is some query about the relevance of PAL if it is not independent of body weight.²⁶ Similarly, AEE is influenced by body weight (energy

expenditure is generally higher for the larger person moving at a given speed) and by the economy or efficiency with which a movement is performed.⁴ Furthermore, because AEE is an average value for the 7–14 days over which the DLW method is utilized, it provides no information on the type, intensity, or duration of physical activity that comprised the AEE. To overcome both the body size and efficiency differences and enable AEE values to be compared between individuals, Weinsier et al.²⁷ devised an index, the activity-related time equivalent (ARTEEE), of the amount of time a person spends at a level of energy expenditure equivalent to a reference task (such as walking at 5 kph) or set of activities (such as standing, walking, or running circuit). The index is calculated using the equation

$$\text{ARTEEE index (min/d)} = (\text{TEE [kcal/d]} \times 0.9 - \text{RMR [kcal/d]}) / (\text{reference activity EE [kcal/min]} - \text{REE [kcal/min]})$$

Despite the validity of this index, because it is based on the DLW technique, the authors recognize its usefulness will be limited to studies of small groups.⁴ However, there is the possibility of using a similar approach to “calibrating” accelerometer data, discussed later in the chapter.

The maximum performance in endurance activities depends on the availability and convertibility of energy.²⁸ It is recognized that there must exist a ceiling, or upper limit, to the TEE and that this ceiling in humans may be set by maximal daily energy intake.²⁹ In addition to maximal rates of food intake and digestion, Hammond and Diamond³⁰ identify maximal sustainable energy expenditure is limited by rates of O₂ uptake and distribution, metabolite removal, and energy utilization by end organs. The most likely limiting factor is end-organ use, the work levels that can be maintained by our musculature. It was estimated from factorial analyses by Brody³¹ that heavy labor could increase the TEE to approximately four times the metabolic rate (BMR). These values were confirmed with DLW studies showing that while the upper limit to sustainable TEE in the general population is 2.2–2.5 times BMR, well-trained athletes who consume large quantities of food while training and competing can maintain energy balance while still expending 4–5 times BMR.³² Higher levels of daily energy expenditure are possible; however, these are not sustainable and the participant will be in negative energy balance and possibly even in a negative nitrogen balance. The consequence is a loss of weight, a higher proportion of which over time will be lean body mass.³³ It has been speculated that it is difficult to ingest and digest enough food during prolonged exercise in excess of 4780 kcal/day.^{34,35} However, Eden and Abernathy³⁶ reported that a male ultraendurance runner covering 1005 km over 9 days (111.7 km/d) consumed 5975 kcal/d. Similarly, Gabel et al.³⁷ reported the average daily energy intake of two elite male cyclists covering 3280 km in 10 days (Pony Express route) was 7122 kcal/d.

It is important to acknowledge that in addition to the exercise intensity, the energy expenditure in exercise is related to body size and the type of exercise being performed. The DLW technique has provided an opportunity to investigate energy expenditure during multi-day endurance events such as cycle tours and running events, as well as in hostile environments (treks in Antarctica or climbing

Mt. Everest, for example) and in military exercises. Westerterp et al.³⁸ measured DLW using a 6- to 7-day protocol, three times in four elite cyclists (69.2 ± 5.9 kg) during the 22-day Tour de France and found average daily TEE to be 7000–8600 kcal/day, which equates to 3.6–5.3 times the RMR (that is, a PAL range of 3.6–5.3).³⁸ Over the 3826 km race, participants were reasonably weight stable; weight decreased on average 1.4 kg. In another study, Rehrer et al.³⁹ used DLW to measure the TEE in five cyclists who completed the 6-day, 10-stage, 883 km Tour of Southland. The average TEE was 6550 kcal/day, but as this cohort were on average 14.6 kg heavier (and thus had a higher RMR) than the cyclists measured by Westerterp et al.,³⁸ the average PAL was lower at 2.39. DLW has also been used in ultraendurance running events. A 2-week DLW study was undertaken in weeks 2–4 of a 14,964 km run around the coast of Australia; the 63 kg male completed the distance in a record 195 days, running on average 76.74 km per day.⁴⁰ During the study period, body weight decreased by 1.5 kg and over the 195-day run, body weight loss was only 1.0 kg suggesting there was neither a significant nor a persistent energy deficit. In this participant, TEE was 6321 kcal/d, and using an estimated RMR, PAL was 3.96. Fudge et al.⁴¹ used a 7-day DLW protocol in nine elite Kenyan endurance runners (56.0 ± 3.4 kg) during a precompetition training phase. Weight loss was not statistically significant (0.3 ± 0.8 kg) and TEE was ~3500 kcal/d, with an average PAL of 2.3. Hoyt et al.⁴² used DLW to measure 23 Marines (79.8 ± 1.3 kg) during 11 days of severe cold-weather mountain training; the average TEE was 4924 kcal/d with a PAL of 2.8 ± 0.2 . Reportedly during the first 4 days of the exercise, the Marines were physically active for 17.93 ± 0.22 hours/day and during this time TEE was 7131 kcal/d with a PAL of 4.0 ± 0.2 . However, during the 11 days there was a loss of body weight of -2.48 ± 0.25 kg. Similar TEE values have been reported in other military cohorts,^{43,44} and weight loss is common. Thus, although the energy expenditure is lower than seen in studies of athletes, energy intake is not well matched. Finally, in possibly the most extreme test of human energy expenditure, a DLW study was undertaken of two men (89.9 kg and 69.0 kg) who pulled sledges initially weighing 222 kg for ~10 hours/day over 95 days in temperatures ranging from -10°C to -55°C , covering a distance of 2300 km across Antarctica.⁴⁵ The reported TEE in the first 50 days (multiple DLW doses were administered) was 8485 kcal/d and 6955 kcal/d for the heavier and lighter man, respectively. Because energy intake was on average 5090 kcal/d, both men lost more than 25% of body weight. The highest TEE values were 10,660 kcal/d and 11,640 kcal/d for the two men; these were recorded between the 20th and 30th days of the expedition, during which time the sledges were pulled for 12 hours/day up hills of 50 m to > 3000 m in altitude. The PAL values reached 6–7, although these were not able to be sustained due to the rapid weight loss.³³ These are the highest values reported in the literature using DLW; however, these are lower than the 14,980 kcal/day (8 times BMR) predicted by Davies and Thompson⁴⁶ for ultraendurance running.

These DLW studies provide us with objective gold-standard data and a “reality check” as to what the highest levels of sustainable and short-term (in terms of days to months) levels of daily energy expenditure that are physiologically possible

in athletes. Any measurement tool that provides values beyond 4–5 PAL in weight stability, or 6–7 PAL in any situation, is most certainly erroneous.

6.3.1.2 Heart Rate Monitoring

6.3.1.2.1 *Measuring Energy Expenditure*

The use of heart rate (HR) monitoring to estimate energy expenditure is based on the assumption that there is a linear relationship between HR and oxygen consumption (VO_2).⁴⁷ Although there is considerable between-individual variability in the slope of the HR- VO_2 relationship reflecting differences in movement efficiency, age, and fitness, the linear relationship holds well within an individual across a range of submaximal aerobic exercise tasks.^{48–50} Consequently, when an individual or group regression line has been determined, HR can be used to estimate an individual's oxygen consumption and in turn energy expenditure in free-living conditions. Since the late 1970s, the development of portable HR monitor devices has broadened the potential usefulness of this relatively low-cost technique for quantifying daily energy expenditure in real-world situations.⁵¹ HR monitors now with increased memory storage and downloading facilities provide the means for recording average HR data per 5 seconds or per minute for over a week continuously.⁵² The advantage of HR monitoring at the individual level is the ability to calibrate the monitor to each individual. Individualized HR- VO_2 regression equations provide greater accuracy as they account for individual differences in health and fitness. An individualized calibration equation can be developed if the athlete completes a submaximal treadmill or ergometer test that reaches 80–85% of an individual's age-predicted maximum.⁵³ A wide range of HR data ensures that the calibration is accurate for various intensities of physical activity.

However, there are important limitations of the method. First, the HR- VO_2 relationship does differ between tasks, particularly for predominantly upper-body versus lower-body activities.⁵⁴ Consequently, the use of a single regression line derived from one movement profile (for example, running) will not be accurate for activities with very different biomechanics (for example, cycling, swimming, rowing).⁵⁵ Another limitation of this approach when used to measure TEE or AEE over a day or days is that despite there being a close relationship between HR and energy expenditure during exercise, there is little relationship between the two parameters during rest and periods of light activity.^{7,56,57} To overcome this problem, Spurr et al.⁵⁸ developed the flex heart rate (flex-HR) method that utilizes an individually predetermined HR to discriminate between resting and exercise HR. The flex-HR method was validated in adults against whole-body calorimetry^{56,58,59} and against DLW⁶⁰ with good agreement for group comparisons. Livingstone et al.⁶¹ used DLW as the criterion and showed that flex-HR was a low-interference technique for accurately predicting group estimates of habitual TEE in healthy, free-living children. The flex-HR method has been applied to verify the minutes spent in activities of different intensities by Ekelund et al.⁶² and to assess TEE and patterns of physical activity in adolescents. Similarly, Grund et al.⁶³ used the approach to verify the effect of gender on different components of total daily energy expenditure (TDEE) in free-living pre-pubertal children. The accuracy of the method depends on the appropriateness of an

individually predetermined HR that can be used to discriminate between resting and exercise HR. Li et al.⁵⁴ suggest that the relationship between energy expenditure and HR differs between individuals and within individuals on different occasions and that it is therefore necessary to develop individual calibration curves immediately before the HR recording period. Other studies have confirmed this suggestion^{64–66} and established that the flex-HR method of estimating EE is reliable at the population level but not necessarily stable for individuals over time. While the best value of the flex-HR may be participant-specific, it may also depend on the mode of activity used to determine the VO_2 –HR relationship.⁶⁷ A further limitation is that HR may be affected by a range of factors other than physical activity, including temperature, humidity, dehydration, emotion, and fitness, and provides no contextual information on the physical activity being performed.^{7,8} Overall the general consensus is that while the HR method provides satisfactory estimates of average energy expenditure for a group, it is not necessarily accurate for individual participants.

6.3.1.2.2 *Monitoring Training Load*

The real value of the HR method is in determining the intensity of discrete bouts of exercise and the estimation of the energy expended in continuous or steady-state aerobic exercise. The American College of Sports Medicine⁶⁸ recommends that the use of HR to describe exercise intensity be expressed using $\%HR_{\text{Reserve}}$ and/or $\%HR_{\text{max}}$. Exercise intensity can then be classified into six categories from very light to maximal. Using the classification process, it is possible to equate the HR category with its associated $\%\text{VO}_{2\text{max}}$ or $\%\text{VO}_{2\text{Reserve}}$ or metabolic equivalent without actually needing to measure VO_2 . However, it is well recognized that greater accuracy is ensured when the VO_2 –HR relationship is measured for each individual.⁵²

In short, despite being a physiological marker for physical activity, HR can be influenced by a wide range of factors potentially unrelated to the activity being monitored. Therefore, HR can provide an estimate or general picture of physical activity⁷ but particularly when used for longer time frames outside a training or exercise competition situation, energy expenditure estimates are improved if HR monitors are used in conjunction with other devices.

Heart rate is also used to monitor training loads and as a physiological marker of overtraining.⁶⁹ In particular, HR can be used to assess the autonomic nervous system following a training stimulus via monitoring heart rate variability (HRV)^{70–72} and heart rate recovery (HRR).^{71,73} Lamberts et al.⁷³ studied the HRR after exercise and cycling performance in fourteen well-trained cyclists undertaking a 4-week high-intensity training (HIT) phase. It was found that endurance performance (40 km time trial) improved more in the cyclists who demonstrated a decrease in HRR toward the end of the HIT period, indicative that a decrease in HRR could be a marker of an inability to cope with the training load and the accumulation of fatigue.

6.3.1.3 **Pedometers**

Pedometers have become popular for health professionals to encourage sedentary adults to adopt a more physically active lifestyle by providing a “steps per day” goal. While the concept of the pedometer is accredited to Leonardo da Vinci, a number of versions of the device were developed in the seventeenth century to count steps

TABLE 6.1
Average Steps per Day Associated with Activity Level

Activity Level	Steps per Day
Sedentary lifestyle	< 5000
Low active	5000–7499
Somewhat active	7500–9999
Active	10,000–12,500
Highly active	> 12,500

for the purpose of measuring plots of land.⁷⁴ More recently, pedometers became the focus of attention after Japanese researchers recommended a daily step count of 10,000 steps as a threshold for health benefit and cardiovascular disease prevention.^{75,76} Hatano⁷⁵ equated walking 10,000 steps per day with 300 kcal energy expenditure, a daily amount identified in the College Alumnus Health Study as optimal to reduce the risks of an initial heart attack.⁷⁷ However, the rationale for the 10,000 steps has been challenged as a viable recommendation for all people.^{78,79} Based on the equivalence of approximately 1250–1550 steps being taken per kilometer,⁸⁰ 30 minutes of brisk walking per day translates to approximately 3000–4000 steps. Using a range of study findings, Tudor-Locke and Bassett⁸¹ have devised Table 6.1 to categorize activity levels relative to different accumulative step counts.

It is beyond the scope of this chapter to compare the relative merits of the different operating mechanisms of pedometers, but the reader is referred to a number of other publications for these explanations.^{74,82–87} A well-recognized problem with the accuracy of pedometers is that step counts are inaccurate for speeds slower than 60 m·min⁻¹, which could be a problem when using the devices in elderly or infirm populations.^{88–90} Furthermore, pedometers worn by different people often register a different step count for the same number of actual steps taken. One reason suggested for this finding is that the impact of foot strike is not uniform for right and left legs, so the pedometer readings can vary depending on where the monitor is worn.^{91,92} Another factor influencing accuracy is waist girth; for centrally obese persons the monitor may rotate when worn on the waistband.⁸²

Even if the steps are measured accurately by the monitor, the interpretation of the step count needs to be clarified. It is common for step counts to be used to determine distance traveled by a simple multiplication of the number of steps taken by the average stride length. The accuracy of this calculation depends not only on the determination of stride length but also on the pace of walking. Stride lengthens with increases in speed.^{93,94} Therefore, walking faster than normal may cause an underestimation of the total distance walked whereas slower walking will cause an overestimation in distance unless there are commensurate relative changes in stride length and step frequency as speed changes; this is not always the case. Furthermore, if stride length is related to height, differences in height will also have an impact on the number of steps taken per unit distance. Most devices do not account for individual differences in height and particularly leg length and its impact on step counts. We have found in a sample of 205 adults heterogeneous in

height (1.70 ± 0.09 m; range: 1.50–1.92 m) that leg length was negatively associated with the number of steps taken to cover 2 km at a self-selected walking speed, with 30% of the variance in steps taken explained by leg length. However, even after adjusting step count for leg length, self-selected walking speed remains a moderately strong influence on the number of steps taken to travel 2 km (unpublished data). The implications are that steps should not be used as a proxy for distance traveled without undertaking an individual calibration of the pedometer to know how many steps are taken for a given distance; calibrating at a range of speeds may also be warranted.

Pedometers can be problematic particularly for youths and the inquisitive participants who continuously look at the readout and can enthusiastically change typical movements to enhance the increasing step count. Pedometers are often taped shut for this reason, and the first few days of recording are often discarded. The advantage of pedometers is that they are relatively inexpensive, are easy to use, and have output data that can be meaningful to the end user as well as to the researcher. Given that walking is the most common physical activity in both light- and moderate-intensity categories, having a good measure of distance and speed is important.⁹⁵ Additionally, it is important that if pedometers are to be used in an intervention or as a tool to monitor changes in daily physical activity, that the sensitivity of the tool to measure change is high. In a small sample ($N = 9$) of obese sedentary adults, Tudor-Locke and Myers⁹⁶ reported that pedometers were able to track modest increases in walking volume, whereas physical activity diaries were not sensitive to the change in ambulation associated with a walking-based intervention. An unfortunate consequence of the popularity of pedometers has been the distribution of many substandard models by companies within breakfast cereal packs, attached to fashion magazines, or made available through general practice clinics. Without individual calibration at regular intervals, these instruments have little more than gimmick value.

6.3.1.4 Accelerometers

Accelerometers measure the rate or intensity of body movement in terms of acceleration, and this enables a profile of the intensity of movement over time. Most devices incorporate piezoelectric sensors that detect acceleration in one to three orthogonal planes (vertical, anteroposterior, and mediolateral). These sensors respond to both frequency and intensity of movement, and in this way are superior to pedometers and actometers that are attenuated by impact or tilt and only count body movement if a certain threshold is passed.⁹⁷ The method is based on the fact that speed is the change in position with respect to time, and acceleration is the change in speed with respect to time. Acceleration is usually measured in gravitational acceleration units (g ; $1\text{ g} = 9.8\text{ m} \cdot \text{s}^{-2}$). When acceleration is zero, the speed is not changing; however, there may still be movement taking place, but it is just happening at a constant speed. Acceleration is proportional to the net external force involved and therefore is more directly reflective of the energy costs associated with the movement. Consequently, measuring physical activity using acceleration is preferred to using speed. The reader is referred to the following papers that provide good reviews of the technical aspects of the methodology and these devices: Chen and Bassett,⁹⁸ Bouten et al.,⁹⁷ and Plasqui and Westerterp.⁹⁹

The first generation of accelerometers consisted of a single accelerometer placed on the waist, due to the proximity with the center of body mass, or on the ankle or wrist to profile movement of the limbs. The most commonly used models of this kind of device include Caltrac, Tritrac-R3D, RT3, Actigraph (formerly known as Computer Science and Applications [CSA] and Manufacturing Technology Inc. [MTI]), Actical, and Actiwatch. Chen and Bassett⁹⁸ provided the technical details of three of the commonly used devices; information is reproduced in Table 6.2. Bouten et al.⁹⁷ have summarized the frequency and amplitude range required to accurately measure human movement. It was proposed that for accelerometers placed at waist level, a frequency band between 0.3 and 3.5 Hz and an amplitude range of −6 g to +6 g should suffice to capture daily physical activities. Within these ranges, accelerations during low-intensity activities, such as sedentary activities or walking, as well as high-intensity activities or exercise, such as running and jumping, can be measured. Low- and high-pass filters can be used to eliminate those frequencies that are unlikely to arise from human movement, such as high frequencies due to transportation.⁹⁹ As can be seen from Table 6.2, not all models meet the criteria proposed by Bouten et al.,⁹⁷ and so arguably may undermine the reliability and precision of the device, and then adversely affect the accuracy of the resulting energy expenditure measurements.

Advantages of these instruments include their relatively small size and capacity to record data continuously for days or even weeks.^{7,98} Unlike pedometers, there is no obvious feedback provided to the participant wearing the monitor. As accelerometers provide no meaningful readout on the monitor itself, there is less likely to be overestimates of physical activity measures in the same way as is seen in pedometers. Compared to the uniaxial sensors, triaxial devices theoretically provide a more comprehensive assessment of body movements. Further, it has been reported in studies of adults and children that higher precision is achieved using triaxial devices for the estimation of energy expenditure when compared to uniaxial devices.¹⁰⁰ One of

TABLE 6.2
Technical Details of Several Commonly Used Accelerometry-Based Physical Activity Monitors

	Actigraph (MTI/CSA)	RT3	Actical
Manufacturer	MTI	StayHealthy	Mini Mitter
Battery type	Coin cell	1 AAA	Coin cell
Battery life	160 days	30 days	180 days
Epoch	1 s–10 min	1 s or 1 min	15 s–15 min
Number of axes	Uniaxial	Triaxial	Uniaxial
Sampling frequency	10 Hz	Unpublished	32 Hz
Frequency response	0.25–2.5 Hz	Unpublished	0.5–3 Hz
Intermonitor CV	4–5%	4–26%	4–19%
ICC	0.8	0.73–0.87	0.62

Source: Chen, K.Y. and Bassett, D.R., Jr., *Med. Sci. Sports Exerc.* 37, S490–500, 2005.

the methodological issues is that while there is a good linear relationship between accelerometer counts and energy expenditure during walking, there have been some concerns in studies of running activities. Early work by Haymes and Byrnes¹⁰¹ demonstrated that the Caltrac accelerometer was a valid indicator of physical activity during walking but did not adequately discriminate between running speeds of 8–12.8 kph. A decade later, Brage et al.¹⁰² assessed the reliability and validity of the CSA (model 7164) accelerometer (MTI) in a wide range of walking and running speeds in the laboratory and field settings. It was noted that the CSA output rose linearly ($R^2 = 0.92$) with increasing speed until 9 kph but remained at ~10,000 counts · min⁻¹ during running; the consequence is an underestimation of oxygen uptake at speed > 9 kph. Brage et al.¹⁰² proposed that the lack of linearity between the uniaxial CSA output and speed when running was presumably due to relatively constant vertical acceleration in running. In Figure 6.3 (unpublished data), we show comparable results using the Actigraph (GT1M); the slope of the relationship reduces at 8 kph.

Rowlands¹⁰³ compared uniaxial (ActiGraph) and triaxial (RT3) devices and confirmed an increasing underestimation of activity by the uniaxial monitor as speed increased. It was proposed that this underestimation was related to frequency-dependent filtering and assessment of acceleration in the vertical plane only. In contrast, the triaxial output was strongly related to speed, reflecting the predominance of horizontal acceleration at higher speeds. Rowlands¹⁰³ concluded that high-intensity activity is underestimated by the uniaxial device even after correction for frequency-dependent filtering, whereas the triaxial device was not limited in the same way. However, there has been some criticism of studies that seek to compare the superiority of triaxial monitors over uniaxial for the purpose of estimating energy expenditure. In these studies, not only are there differences in the number of axes but also the model of device, and hence the monitor technology differs. To overcome this

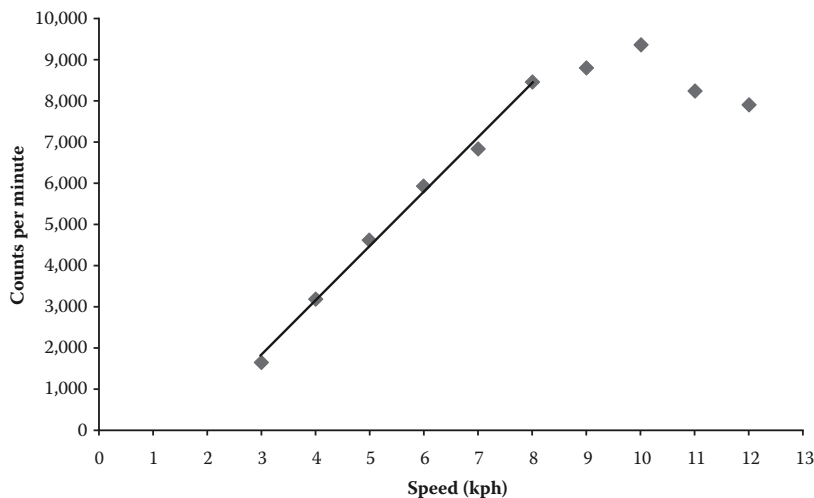


FIGURE 6.3 Average counts per minute of an individual walking (3–6 kph) and running (7–13 kph) using the Actigraph (GT1M).

limitation, Howe et al.¹⁰⁴ has recently used a single device that is capable of measuring and reporting movement in all three axes separately and simultaneously (RT3) to determine whether using three planes of acceleration signals is superior to using only the vertical plane of the same unit for predicting AEE during locomotion and activities of daily living. Compared with vertical-only counts, using the integration of the three axes did not significantly improve the relationship between counts and AEE. However, it is important to note that compared with indirect calorimetry, RT3 overestimated AEE for treadmill activity by 9% and underestimated activities of daily living (ADL) by 34%. The RT3 underestimated activity with greater upper body movements by 24–64%. Compared to DLW over 15 days and using the proprietary algorithms, Maddison et al.¹⁰⁵ found the RT3 underestimated AEE by 15% on average. The authors suggest that while the RT3 may provide a relatively accurate assessment of free-living AEE at the group level, it generally underestimated the activity-related energy expenditure compared to DLW. These studies demonstrate that is not sufficient only to consider the number of axes used; the technology inherent in the device or the data processing may be the source of error. When using multiple regression to devise an AEE prediction equation from participant characteristics and activity counts, many studies do not mention partial correlations for activity counts or the increase in R^2 caused by the activity counts. Plasqui et al.¹⁰⁶ note that age, body mass, and height collectively explained 64%, while the triaxial accelerometer (Tracmor) added only an additional 19% of the variation in TEE. In some studies it is possible that most of the variance is explained by participant descriptors and the accelerometry data may have only marginal additional value. Few studies have provided data to show the ability of the accelerometer to predict individual AEE rather than AEE on a group level only; standard errors or limits of agreement should be presented. Plasqui and Westerterp⁹⁹ outline an important range of issues for consideration when comparing the validity of different accelerometers.

Even without converting the accelerometer counts to energy expenditure, the raw data from the accelerometer can be used to quantify time spent by an individual in activities of different intensities. As shown in [Figure 6.4A](#), the output can be related to standard thresholds for light, moderate, and vigorous intensity movement.^{107–111} However, uniform cutoff points may not be truly representative of the same exercise intensity across individuals. As shown in [Figure 6.4B](#), individuals walking at the same speed (4.8 kph) had an average output (vector magnitude) over 6 minutes of 1778 counts per minute. However, the variance around the group mean indicates a high interindividual variation with a range of 71–129% of the mean value.

Shortcomings of the system are the low sensitivity to sedentary activities and the inability to register static exercise.⁹⁷ Because accelerometry is insensitive to physical activity that does not involve a transfer of the center of mass at a rate relative to the energy expended (for example, weight lifting, walking up a grade, walking while carrying a load), this will result in errors in energy expenditure measurement.^{111,112} It has been demonstrated that both inter- and intramonitor variability exists with monitors; therefore, it is recommended that every laboratory perform trials to identify outlying monitors and satisfy itself that the intermonitor variability is acceptable before use.^{113,114} Further, as outlined above, accelerometers should be calibrated to each individual user.

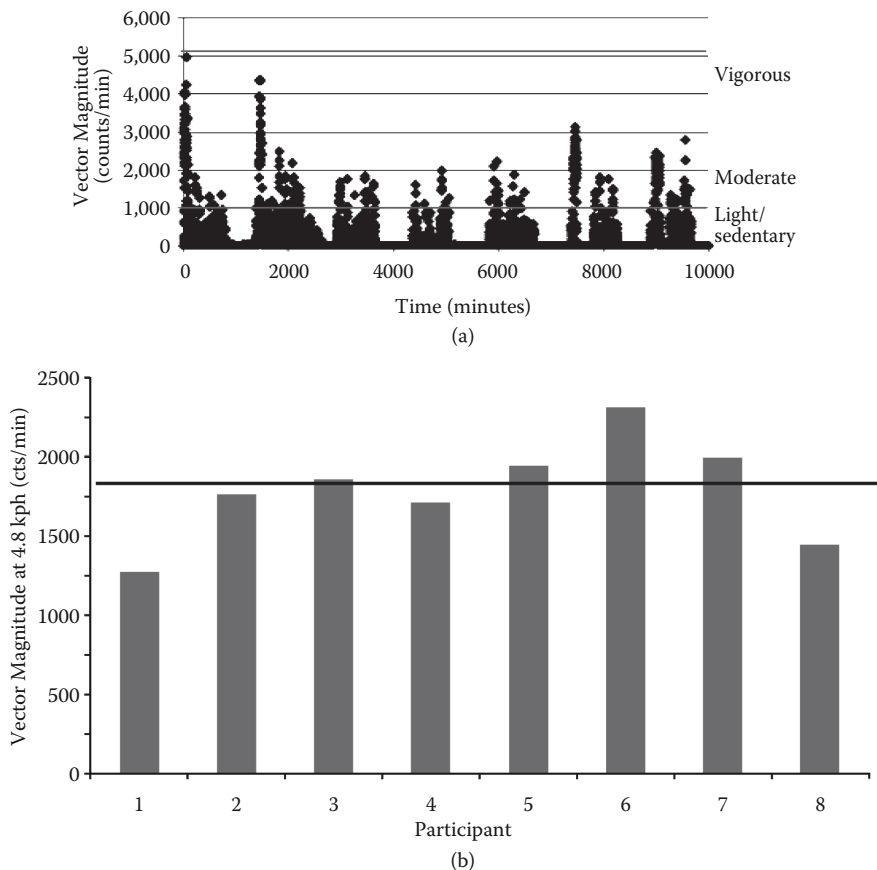


FIGURE 6.4 (a) Raw RT3 accelerometer counts categorized according to activity thresholds; (b) interindividual variability in accelerometer counts when walking at the same speed (4 kph); horizontal line represents the group average.

Accelerometers may also be used as a measure of inactivity. We have previously shown in a sedentary group of men with schizophrenia that accelerometry was a poor measure of activity energy expenditure when compared with the criterion measure of DLW.¹¹⁵ The correlation between the accelerometry output (VM) and AEE was not significant. However, a significant negative relationship was found between inactivity (defined as a VM less than 20 counts/min) and AEE ($r = -0.83$, $p = 0.001$), with minutes spent sedentary explaining over three quarters of the variance in AEE. Therefore, in very sedentary groups the accelerometer data may be a valid measure of inactivity and therefore could be used for research or clinical purposes to quantify the contribution of sedentary behavior to weight gain or medical conditions associated with inactivity, such as cardiovascular disease and diabetes. Further, the effectiveness of interventions to reduce sedentary behavior could also be objectively monitored with accelerometers.

To improve the categorization of movement in recent years, multiple accelerometers have been worn on different body parts (trunk, chest, wrists, legs, and feet)

and whole-body energy expenditure determined from the composite of movements. Recently, devices other than standard accelerometers have been developed, such as the ActiReg¹¹⁶ and the Intelligent Device for Energy Expenditure and Activity (IDEEA),^{117,118} that use multiple sensors to assess both body posture and body movement, which are then translated into energy expenditure. These new technologies have been evaluated in a range of studies with mixed results.

The ActiReg has two body position sensors (tilt switches) and two motion sensors. The state of the tilt switches and motion sensors is checked every 1 second. The sensors discriminate between the four body positions (sit, stand, bent forward, or lie), and between the four states of no motion, motion on either chest or thigh sensor, or both. This gives a matrix of $2^4 = 16$ possible combinations with ActiReg codes from 0 to 15. These codes are linked with an activity factor, from which energy expenditure can be determined. In the first published study using the ActiReg, the calculated TEE did not significantly differ from DLW-measured TEE on a group level in adults, but the individual variation in the difference between both methods was large. Furthermore, the ActiReg underestimated TEE to a greater extent at higher levels of energy expenditure.¹¹⁶ More recently the device has been used to assess energy expenditure in clinical populations.^{119–121} The IDEEA device showed good results under laboratory conditions but require multiple sensors to be attached to the body and have a relatively large data acquisition unit, thereby diminishing wearing comfort. So far, they have not been proven to be superior in the estimation of EE to simpler accelerometers, and further research is required to determine their effectiveness as a measure of daily life AEE.⁹⁹

Rather than using multiple devices that may increase cost, increase the possibility of missing data, and raise the participant and researcher/coach burden, recent studies have focused on the identification of activity types based on the acceleration features measured with a single accelerometer.^{122,123} Bonomi et al.^{124,125} measured activities of daily living with a single accelerometer during daily life in a population of healthy adults, and utilized a decision tree to identify the different activity types performed. The decision tree evaluated attributes of the acceleration signal. Using DLW as the criterion measure, the identification of types of physical activity, such as lying, sitting or standing, active standing, walking, running, and cycling, performed during the day combined with a simple methodology to define activity type intensity improved the estimation of TEE, AEE, and PAL compared with activity counts.

The ongoing technological advancements in accelerometer design and data processing approaches are likely to see this methodology become a tool of choice for assessing both physical activity and energy expenditure in the future.

6.3.1.5 Combined Approaches and New Devices

The idea that HR monitoring and accelerometry be used simultaneously to assess energy expenditure has been suggested by several researchers.^{100,126–128} The major underlying rationale for using both techniques simultaneously is that the accelerometer is used as a backup measure to verify that elevations in HR are indicative of physical activity.¹⁰⁰ HR monitoring and accelerometry both encompass several very different limitations. The heart rate–energy expenditure relationship is affected by age, sex, training state, stroke volume, hemoglobin concentration of the blood

and its O_2 saturation, mental stress, ambient temperature, hydration, and quantity of muscle mass involved in the activity. The limitations of the accelerometry devices are biomechanical in nature and include issues with graded and load-bearing activity. Accelerometers lack both the ability to account for changes in the grade of the exercise surface and any changes in load carried by the user.¹²⁶ Since the limitations of each technique are unrelated, the combination should theoretically yield a more precise estimate of energy expenditure than either used alone.^{126,127} Another reason for combining the techniques would be to employ HR monitoring for the assessment of physical activity energy expenditure and use accelerometry to assess total daily movement and calculate activity patterns that make up the AEE. The heart rate- VO_2 relationship is consistently linear only during dynamic muscle exercise of moderate to high intensity,¹²⁹ thereby supporting the use of HR monitoring for the quantification of physical activity energy expenditure (PAEE) and not the lower-intensity activity that occurs in daily living. In contrast, accelerometry is very limited in assessing AEE yet appears to accurately quantify low levels of activity or sedentary behavior.¹²⁸ A range of devices combining HR and movement monitoring capabilities are now on the market (Actiwatch/Actiheart/Actiband; ActiTrainer).

Other devices are being developed that extend beyond the combination of heart rate and movement monitoring. The SenseWear Armband (www.bodymedia.com) is a multiple sensor device collecting data from a skin temperature sensor, near-body temperature sensor, heat flux sensor, galvanic skin response sensor, and a biaxial accelerometer. These signals are combined to assess the type and intensity of an activity. Together with information about gender, age, height, and weight, energy expenditure is estimated using activity-specific, proprietary algorithms. According to the manufacturer, the device is clinically valid in subjects between 7 and 65 years of age who are engaged in resting, ambulatory, stationary biking, motoring, and weight-lifting activities. These tools are largely marketed to the weight-management rather than athletic market.

For the athlete, various companies are developing tools for monitoring exercise, not all of which have been independently evaluated. Three of the more prominent companies are Polar (www.polar.fi), Suunto (www.suunto.com), and Garmin (www.garmin.com). There is a growing range of products that, in addition to heart rate monitoring, enable data collection of numerous mechanical variables: distance, speed, altitude, cycling power output, running pace, and cadence, as well as other compass features, altimeter features, GPS features, and cycling and running features. It is beyond the scope of this chapter to review each of these new technologies. However, it is evident from the range of new tools currently available and under development that there will be a wide array of possible assessment tools available in the future. More research is of course required to evaluate the relative merits of each of these new technologies.

6.3.2 SUBJECTIVE APPROACHES

Subjective approaches include direct observation, activity diaries, and physical activity questionnaires and interviews. A major shortcoming of subjective methods is that the accuracy of information collected is influenced by a range of factors, including

the ability of the athlete to recall information retrospectively and the perception of the participant regarding the nature of the responses required or expected. Borresen and Lambert³ compared what athletes reported doing in training and what was actually completed. Twenty-four percent of the participants overestimated the duration of training they were doing, and 17% underestimated their training duration. Because this margin of error in self-reported data may significantly affect the prescription of training, it has been suggested that the error be accounted for or, where possible, physiological measurements be used to corroborate self-reported data.¹³⁰ The use of data collected by questionnaires to quantify exercise load is also limited by inadequate reliability and validity compared with laboratory measures; for example, the reliability decreases as the time between the activity and recall increases, because this is dependent on human memory.¹³¹

Interview-administered approaches are generally superior to self-report questionnaires and the validity of such approaches may be severely compromised in younger athletes who typically have more problems in recalling physical activity. Direct observation was one of the earliest methods of physical activity assessment used and while it is often categorized as an objective approach, unlike other objective tools (such as timing lights to measure speed, filming for video analysis), it relies predominantly on the coach's perceptions of the athlete's level of effort or quality of training or performance. Perceptions by coaches and athletes of the same training have been studied by Foster et al.,¹³² who showed significant differences between the training that the coaches prescribed and the training the athletes actually completed. Therefore, the extent to which training can be quantified based on direct observations has been questioned.³ Further, because this method requires the presence of an observer at every training session, which may be impractical or impossible, the amount of data able to be collected in order to monitor and evaluate training accurately may be inadequate.¹³⁰

The validity of data at the individual level collected using subjective measurement approaches is commonly not as strong as using a more objective approach such as accelerometry or the doubly-labeled water technique across a 7- to 14-day period. However, the validity of data at the group level may be higher and the low cost of most subjective methods creates an advantage for large, population-based studies.^{7,133} In addition to the low relative cost, a major advantage of subjective approaches is the ability to derive information about specific physical activity behaviors.⁸ A brief overview is provided of a number of the subjective approaches to assess physical activity.

6.3.2.1 Direct Observation

Direct observation generally encompasses a significant element of subjectivity in scoring activity participation and the subsequent interpretation of data. The presence of an observer can cause the participant to react differently, and this may result in an activity being over- or underestimated. Similar changes to behavior can be seen when equipment—for example, a video camera—is used to record activity. However, such differences are likely to diminish over time and therefore be normalized for the period of observation.

Familiarization periods can be used to allow participants time to adapt to the presence of an observer and subsequently diminish the error from participant reactivity.

Observer bias is also a concern when using direct observation and can be influenced by the participant's gender, the purpose of the study, or the behaviors of surrounding peers.^{133,134} The approach is not suited for use in free-living activities due to the burden on the observer; however, a major advantage of direct observation is its suitability for small numbers of participants over short periods of time, assuming the availability of trained observers.⁸ The method has also provided important contextual information—for example, regarding activity level and distance covered during match conditions—which may not be obtainable with other physical activity measurements.¹³⁵

6.3.2.2 Physical Activity Records or Diaries

Physical activity records, or diaries, can provide detailed accounts of activity types and patterns through descriptions of activity type (such as walking, watching television), purpose (for example, exercise, occupation, housework), duration (minutes or hours, for example), intensity (light, moderate, vigorous), and body position (reclining, sitting, standing, moving, and so forth).² Considerable detail may be recorded in relatively short time intervals, typically every 15 minutes. This information can be collated to determine time engaged in various activities and/or translated into predictions of energy expenditure using metabolic equivalent (MET) values for each task and intensity level.² Typically, this requires the use of the compendium of physical activities and a coding scheme that is based on a five-digit code that describes physical activities by major headings (occupation, transportation, and so on) and matches the specific activities within each major heading with a corresponding intensity (displayed as METs). The compendium of physical activities was created in 1989 and updated in 2000. It is often used in physical activity records because of its easy translation across studies.¹¹ However, the amount of detail necessary to complete the records over small amounts of time can create a high administrative burden for both the participant and the investigator.² Because of this burden, it has been suggested that the physical activity records primarily be used to monitor high risks of energy imbalance (such as obesity, metabolic syndrome) or when detailed physical activity information is needed in relation to health conditions.²

A disadvantage of activity diaries is that individuals are required to record physical activities in blocks of time (ideally to account for each 15 minutes) across multiple 24-hour periods. Diaries have been used in different populations but have a high participant burden.

6.3.2.3 Physical Activity Questionnaires

Physical activity questionnaires are the most commonly used physical activity assessment tool.¹³² There are three main types of questionnaires: global, recall, and quantitative. A global questionnaire is a brief survey that is easy to complete in relatively short periods of time but does not provide explicit detail of the physical activity. It is useful when a group is being generally categorized, such as “active” or “inactive.” Recalls are longer and more detailed accounts of physical activity and are typically considered a separate assessment tool. Quantitative questionnaires are long and detailed, providing information about the frequency and duration of specific activities over extended periods of time (such as year, lifetime). Although a heavier burden on the participant, the quantitative questionnaire is good for assessing

lifestyle factors or physical activities that are associated with disease or chronic health.² Physical activity questionnaires vary in terms of amount of detail provided, length of time assessed, and the extent of supervision during the completion of the questionnaire.¹³¹ The most common questionnaires are the Baecke, Tecumseh, or Minnesota Leisure Time Physical Activity Questionnaire, or variations thereof. A systematic review of physical activity questionnaires identified four physical activity questionnaires that had the basic research design components necessary to estimate activity energy expenditure, which included the Tecumseh and Minnesota Leisure Time Physical Activity Questionnaire (alone and in combination), as well as the Questionnaire d'Activité Physique Saint-Etienne.²⁰ The Tecumseh questionnaire involves individual interviews pertaining to the estimated hours per week spent in sport, home repair, sleeping, eating, quiet leisure time, and all remaining activities.¹³⁶ The Minnesota Leisure Time Physical Activity Questionnaire assesses daily physical activity completed during leisure time and household activities for a period of 12 months.¹³⁷ Both studies, as well as the Baecke questionnaire and others, have been validated using doubly-labeled water as the criterion measure for assessing physical activity in various populations. The correlation to doubly-labeled water is low, specifically at the individual level, with group levels reaching only moderate correlations to the criterion measure.^{20,133} Additionally, longer tests can confuse or bore the participant, resulting in low validity and test–retest variability.^{138,139} Simpler tests, such as the Baecke and Godin questionnaires, have test–retest correlations as high as 0.81 and have had better success, relative to a Caltrac motion assessment of concurrent validity.¹³¹

Physical activity recalls are a type of physical activity questionnaire and include approximately 7–20 items to identify details about physical activity.² The recall is completed over at least a 24-hour period, with 7-day recalls typically used to assess physical activity.²⁰ Because of the length of time that is necessary for a person to recall, physical activity recalls are more valid when administered as an interview (either by telephone or in person). Similar to the physical activity records, information provided by a physical activity recall can identify exercise intensity levels through METs. However, recalls tend to overestimate vigorous physical activity while simultaneously underestimating the amount of time spent completing habitual activities of daily living.² In spite of this error, physical activity recalls have been used to determine if a person is meeting activity guidelines.²

Self-report questionnaires represent the most commonly employed methods of physical activity assessment and such instruments are suitable for individuals over approximately 10 years of age (assuming a standard level of comprehension and literacy). Parent, teacher, and/or health professional proxy report approaches are required when working with younger children or older adults, respectively. Although numerous self-report measures are available, they vary substantially. A particular strength of questionnaires is their ability to capture the type, duration, and frequency of daily physical activities, which is not possible using the DLW technique or sums of HR over extended periods of time. However, there is a need to devise and validate appropriate questionnaires for the purpose of both evaluating physical activity and predicting total and activity energy expenditures. Questionnaires are most valuable when used simultaneously with objective energy expenditure measurement approaches.

Population-based surveys are commonly self-report instruments completed by respondents or based on telephone responses to questions. Some of the strengths of population surveys include the large numbers, the potential to access representative samples, and the consistency of questions over time when the surveys are repeated. However, weaknesses include the reliance on self-reported data, restriction to collect only data on certain types of physical activity behavior and energy expenditure, and a lack of coverage for some population subgroups. Given these shortcomings, the validity of the data and confidence in the results may be questioned.

6.3.2.4 Self-Report Physical Activity Questionnaires

Questionnaires are commonly used to evaluate the frequency and duration of specific physical activities. The method is relatively economical and can therefore be used to assess large numbers of participants. Table 6.3 provides an overview of the strengths and limitations of this methodology with specific reference to individual questionnaires.

The validity of some self-report physical activity data and energy expenditure values derived from this data has been questioned. For example, some groups may overreport the volume of physical activity they engage in. The accuracy of one’s ability to recall physical activity also varies according to age, gender, body size, level of education, and household income. It is also unclear which aspects of misreporting may be most problematic: the duration, intensity, frequency, or type of activity. Any miscalculation of the total volume or intensity of physical activity may have different implications in the determination of, for example, the dose–response relationship between physical activity and health. It is highly desirable to include objective measures with self-report instruments to minimize intentional or unintentional misreporting of physical activity. If a combined approach is not possible in

TABLE 6.3
Strengths and Weaknesses of Self-Report Questionnaires

Strengths	Limitations
<ul style="list-style-type: none">• Able to measure large numbers of participants at low cost• Theoretically, the recall process does not alter behavior• Variety of dimensions of physical activity can be assessed• Extrapolation to energy expenditure estimates can be made• Suitable for a wide variety of populations as only a pen and paper are required• Measurement tool can be adapted to suit the population• It is possible to compare results from different locations when the same instrument is used (e.g., International Physical Activity Questionnaire—IPAQ)	<ul style="list-style-type: none">• Recall limitations for some populations (for example, children and the aged); therefore, cognitive demand needs to be considered• Semantics used may be a problem in some settings; for example, terms may be ambiguous to some (such as “physical activity,” “moderate intensity,” “energy expenditure”)• Dependent upon response rates and ability of participants to follow instructions• Completeness of questions answered• Activity choices listed in questionnaire may not be relevant for some certain populations• Minimum amount of detectable change may not be well defined; sensitivity

the whole cohort, it is recommended that both measurement approaches be used in a representative subsample.

In many settings, data from physical activity questionnaires have been used to quantify energy expenditure. The metabolic equivalent (MET) has been widely used to provide a common descriptor of intensity of physical activity in multiples of RMR.

One MET equates with the oxygen consumption (VO_2) required at rest, assumed to be 3.5 mL/ O_2 /min/kg body weight. The MET is also defined as the ratio of work metabolic rate to a standard RMR of 1.0 kcal (4.184 kJ)/kg/h. Comprehensive lists of energy expenditure estimations for numerous physical activities have been developed and published in a compendium.¹¹ Typically, an estimation of daily energy expenditure can be gained by converting time spent in physical activity to energy equivalents using the compendium.

The precision of this factorial method^{140–142} in quantifying human energy expenditure is influenced by two main factors. First, physical activity estimates are only as good as the information recorded, and therefore the accuracy of an individual's recall of the physical activities completed is a major influencing factor. Secondly, energy expenditure estimates will be influenced by the accuracy of the assigned MET level and the underlying premise of the factorial system; that is, how consistent is the assumed resting value of 3.5 mL/ O_2 /min/kg body weight across individuals of different sizes and shapes?

It is important to highlight that the compendium was not developed to determine the precise energy cost of physical activity within people.^{11,143} Rather, the approach was developed to classify activity and standardize the MET intensities in population health research. The MET system has been widely used by researchers, clinicians, and practitioners to identify and prescribe physical activities. There is increasing evidence that estimates of activity energy expenditure using the factorial system may be inaccurate across individuals of different body mass and body fat categories. In a heterogeneous sample of 769 adults (18–74 years of age, 35–186 kg) who were weight stable and healthy, albeit obese in some cases, the 1 MET value of 3.5 mL/kg/min overestimated the actual resting VO_2 value on average by 35% and the 1 MET of 1 kcal/kg/hr overestimated resting energy expenditure by 20%.¹²

6.4 CONCLUSIONS

Accurate measurement of physical activity and quantification of energy expenditure can be challenging with the vast array of approaches available. All methods have inherent strengths and weaknesses, and it is therefore important that one understands these strengths and limitations and makes a selection based on the appropriateness of an instrument or instruments to meet his or her specific needs. Subjective approaches, including diaries and recall, have the potential to provide rich descriptive data but are heavily reliant on the memory of the individual and may be prone to overreporting some activities and underreporting others, such as incidental physical activity. More objective methods such as accelerometers fail to adequately assess some modalities of activity such as cycling and swimming, or are impractical in a sporting context.

With the availability of more sophisticated technology, we are certain to see further growth in the range of tools to assess physical activity levels and energy expenditure. To do justice to this growing field we need to be sure of the reliability and accuracy of the tools used to collect the data we are intending to measure and to know how to interpret any change or difference we may see. When there is the opportunity to measure physical activity with research quality tools, a multimethod approach enables a more complete picture to be gained. It is important that physical activity as well as inactivity is monitored; that exercise intensity as well as total dose are considered; that reliability of instruments are checked regularly to account for signal decay; and, where possible, that instruments are calibrated to the individual.

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7 Molecular Aspects of Physical Performance and Nutritional Assessment

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7.1 INTRODUCTION

From the dawn of history, athletes paved the road for humanity to enhance human physical performance. Through improved and rigorous training methods, controlled surroundings, and precisely measured food intakes, they have attained the highest possible levels of human achievability. Traits are affected by genes as well as by environmental factors. Variations in any trait/phenotype (athleticism among them) are expressed as the result of genotype by environmental interactions ($G \times E$). Historically, most of the performance-enhancing methods targeted environmental factors, since controlling the genetic variability was not an option within the reach at that time.

Recently with the completion of human genome sequencing projects and the full sequencing of entire genomes of other model organisms, such as baker's yeast (*Saccharomyces cerevisiae*), fruit fly (*Drosophila melanogaster*), zebra fish (*Danio rerio*), and roundworm (*Caenorhabditis elegans*), and the initiation of sequencing projects for additional organisms such as bovine and swine genomes (*Bos taurus*

or the domestic cow), an unprecedented abundance of information about different genomes became publicly available. Our understanding of genes, their products, and their functions has been enhanced dramatically in the past two decades. Comparative genetics, bioinformatics, and sequence alignment methods have been successfully used to annotate genes and discover their novel functions. Conditions affecting transcript abundance coded by these genes can be easily analyzed presently using state-of-the-art, real-time polymerase chain reaction (RT-PCR) machines and changes taking place at certain genomic loci or even the entire genome can be tracked with chromatin immunoprecipitation (ChIP) or ChIP to Chip methods, respectively.

These advancements in our understanding of genomes and proteomes went hand in hand with similar research methods trying to find empirical uses of this acquired knowledge through biotechnological methods in almost all branches of life sciences spanning physiology, medicine, nutrition, and sports. For the first time in history we are facing ethical dilemmas of our increased ability to control and enhance human performance. Improvements in gene therapy techniques and the successful horizontal gene transfer (from person to another within the same generation) as compared to vertical passage of genes from generation to generation, started adding new challenges to our comprehension and defying our traditional concept of sportsmanship and fair competition. This chapter discusses and introduces the readers to some of these current issues.

7.2 PHYSICAL PERFORMANCE GENES

Evidence has recently linked genetic variation to athletic ability. Early observations of genetic variations and DNA polymorphisms and their association with elite athlete status and training responses are being confirmed every day. A literature search reveals that almost all of desired physical performance characteristics are influenced somehow by the genetic component. More than eighty different genetic markers (located within autosomal genes, Y chromosomes, and mitochondrial DNA) are linked to elite athlete status and might explain the variations among individuals in their response to training.^{1,2}

7.2.1 CARDIORESPIRATORY AND ENDURANCE GENES

Endurance sports (such as swimming, running, and rowing) require athletes to perform low- to medium-intensity work over a long period of time. These types of sports differ from the explosive form of energy and muscle strength needed in power sports (such as shot puts, weight lifting). Training methods target enhancing and developing efficient energy production systems needed by these athletes to keep the demand during competitions and events. The athlete's heart (modulated heart size) is among the observed adaptations to such training conditions. Yet this trait shows considerable variation among athletes and trainees. Recently, Karlowatz et al.³ reported that this adaptation correlates with genetic polymorphisms in insulin-like growth factor 1 (IGF1). The analysis of *IGF1* gene, IGF1 receptor (IGF-R), myostatin (MSTN), and mutation screening of the *MSTN* gene in 110 elite athletes engaged in endurance training and their relation to left ventricular mass (LVM) revealed that polymorphisms

in the IGF1 and the IGF1-R gene, such as G to A substitution at position 3174, has significant relation to left ventricular hypertrophy (LVH) in male athletes. The team also confirmed the effect of one additional unnoticed polymorphism (allele deletion shifting AAA to AA) targeting the first intron of the *MSTN* gene, which increases the myostatic effect.

In the past decade, much of the attention to polymorphisms and endurance training associations was drawn to the angiotensin-converting enzyme (ACE), which is part of the renin-angiotensin system (RAS). *ACE* plays a key role in circulatory homeostasis by degrading vasodilator kinins and generating angiotensin II, a growth factor. Intron 16 of the human *ACE* gene has been linked to endurance training response by two polymorphisms.⁴ One is an insertion (I allele) and the other is a deletion (D allele) of a 287 base pair (bp) fragment. The deletion polymorphism is associated with lower serum and tissue ACE activity while the I allele is associated with endurance performance and found with more than usual frequency in elite athletes.^{4–20}

In a study conducted by Karjalainen et al.,²¹ the LVM was measured in eighty young elite endurance athletes (age 25 ± 4 years) screening the angiotensinogen (AGT), angiotensin-converting enzyme (ACE), and angiotensin II type 1 receptor (*AT1*) genes for the M235T, insertion/deletion (I/D), and A1166C polymorphisms, respectively. The study concluded that the M235T polymorphism affecting angiotensinogen gene was significantly associated with the variability in LVH induced by endurance training and athletes carrying homozygous T alleles developed the largest hearts. Both ACE and AT1 polymorphisms showed little association with LVH variability.

Recently, the peroxisome proliferator-activated receptor- α (*PPAR- α*) gene was suggested to also be involved in LVH. Originally, this receptor regulates genes involved in fatty acid oxidation in heart and skeletal muscles. One polymorphism in this receptor targeting intron 7 (with a G to C change) is associated with left ventricular growth in response to exercise. Endurance-oriented athletes, power-oriented athletes, and athletes with mixed endurance/power activity were tested for this polymorphism.²² An increasing linear trend of C allele was found with increasing anaerobic component of physical performance. The GG homozygotes were more prevalent within the endurance-oriented athletes. The second interesting observation that was found in this study is the connection between *PPAR- α* gene variant and fiber type composition. Muscle biopsies from m. vastus lateralis that were analyzed revealed that GG homozygotes have significantly higher percentages of slow-twitch fibers than CC homozygotes.

The above results show the importance of polymorphisms affecting different signaling pathways (receptors and growth factors) on variant degrees of physiological hypertrophy of athletes. Some of these polymorphisms do confer an advantageous effect, most likely mediated via improved muscle efficiency with secondary benefits in terms of conservation of nonfat mass.

7.2.2 MUSCLE GROWTH AND REPAIR GENES

Muscle growth and strength is an essential trait in sports and usually constitutes the first target for any training program. The desired rate of achieved growth and

strength differs from one sport to another and depends on the targeted muscles. Muscle development and growth under training is affected largely by certain genes. Most studies associate the ACTN3 genotype with this trait and knockout studies carried out in mice gave conclusive evidence for such association.^{23–49} The actin-binding protein α -actinin-3 (ACTN3) is expressed only in fast-twitching fibers (Type 2) in skeletal muscles and evidence is provided that an early termination codon within the coding region of this protein leads to a R577X phenotype. This phenotype is prevalent in at least 18%, 25%, and less than 1% of healthy white, Asian, and African Bantu individuals, respectively.⁵⁰ The presence of untruncated ACTN3 protein generates forceful contractions at high velocity compared to the absence phenotypes. The presence of this protein is more frequently found in elite power athletes.⁴⁸ A study conducted on 107 elite athletes (males and females) specializing in sprint/power events showed significantly higher frequencies of 577R allele than the control individuals. As mentioned earlier, studies with the mice knockout model confirmed these observations.³¹ The absence of α -actinin-3 resulted in a reduced force generation, reduced fast-twitched fiber diameter, increased activity of multiple enzymes in the aerobic metabolic pathway, and enhanced recovery from fatigue, suggesting that the null phenotype in mice and humans is more suited for endurance sports compared to strength performance. The developed model in these aforementioned studies suggests also a role for myogenin (muscle regulatory factor) as a positive regulator for muscle growth compared to α -actinin-3, which acts as a negative regulator. A genetic test for the presence/absence of 577R alleles is currently available commercially to test teenagers and junior athletes in order to help them in deciding which sport would best suit them and support their maximum achievability.

Other genetic variants and polymorphisms within muscle growth and strength regulators (positives and negatives) are also known. Polymorphisms that affect IGF-1, fibroblast growth factor (FGF), hepatocyte growth factor (HGF), mechano growth factor (MGF), and myostatin are also known to affect muscle growth and regeneration after exercise, and readers are directed to more detailed reviews to explore the functions of these factors.^{1,3,51–59}

7.2.3 PAIN RELIEF GENES

Pain is frequently associated with rigorous training that results in damages to skeletal muscles manifested in delayed muscle pain. Clarkson et al.⁴⁶ proposed that variations in *ACTN3* and *MLCK* genes might explain the large variability in the response to muscle-damaging exercise. Specific single nucleotide polymorphisms (such as C49T and C37885A) in myosin light chain kinase (MLCK) showed a greater loss in muscle strength and a greater increase in blood creatine kinase (CK) and myoglobin (Mb) in response to eccentric exercise. Their results indicate that variations in gene coding for these specific myofibrillar proteins influence phenotypic responses to muscle damage, hence the accompanied pain.

Pro-inflammatory cytokines such as interleukin-1, IL-6, and IL-17 are also known to be involved not only in inflammation but also in the induction and probably the perpetuation of pain.^{60,61} These cytokines exert their biological effects on C-reactive protein (CRP) by signaling through their receptors on hepatic cells and activating

different kinases and phosphatases, leading to translocation of various transcription factors to *CRP* gene promoter and production of CRP protein, which is considered the triggering compound of most chronic diseases and the associated pain.⁶²

More polymorphisms and genetic factors related to pain sensitivity are being identified.⁶³ Subjects with single nucleotide polymorphisms in GTP cyclohydrolase (*GCHI*) and mu opioid receptor (*OPRM1*) genes are known for their higher pain sensitivity and lower pain thresholds.⁶⁴ Studies also reveal that extracellular adenosine 5'-triphosphate (ATP) and its P2 purinergic receptor located on cell surface are involved in neuropathic pain. Polymorphisms that cause changes of this receptor function lead to an increased pain sensitivity induced by the cold stimuli.⁶⁵ Finally, the relationship between functional polymorphisms in dopaminergic genes and sensitivity to pain in healthy subjects was established. The variable number of tandem repeat (VNTR) polymorphisms of three dopamine-related genes were investigated (a 30-bp repeat in the promoter region of the monoamine oxidase-A gene [*MAO-A*], a 40-bp repeat in the 3'-untranslated region of the dopamine transporter gene [*DAT-1*], and a 48-bp repeat in the exon 3 of the dopamine receptor 4 gene [*DRD4*]). The results indicated a significant association between cold pain tolerance and *DAT-1* and *MAO-A* polymorphisms, suggesting that low dopaminergic activity is associated with high pain sensitivity.⁶⁶

7.2.4 FRACTURE REPAIR GENES

Stress fractures (such as affecting mid-tibia, diaphyseal femur) constitute challenging problems in the upper-tier athletes. The demand for continuous training with very little room for prolonged rest periods makes the susceptibility for such injuries higher among this population.⁶⁷⁻⁷¹ Bone tissue development and repair is a complicated process under the control of different types of proteins and enzymes (such as IGF, mineralization proteins, regulatory factors). The repair process requires the activation and coordination of several pathways leading to the transformation of mesenchymal precursor cells to osteoblasts. Recently, a transcription regulator, CBP/p300-Interacting-Transactivator-with-ED-rich-tail-2 (CITED2), which suppresses genes involved in angiogenesis, osteogenesis, and extracellular matrix (ECM) remodeling, was identified.⁷² In fractured mandible, CITED2 expression was inversely related to the expression of matrix metallo-proteinases (MMP-2, MMP-3, MMP-9, MMP-13) and the overexpression of CITED2 in osteoblasts inhibited the activity of these metallo-proteinases. This suggests that CITED2 has a critical function as an upstream regulator of fracture healing and that the suppression of CITED2 early after fracture may allow for an optimal initiation of the healing response.⁷²

In a similar study, the role of Akt protein kinase was scrutinized. Mukherjee and Rotwein⁷³ demonstrated that dominant-negative Akt cells prevented osteoblast differentiation in a similar manner to IGF binding protein IGFBP5, a protein crucial for normal skeletal development and bone remodeling. An adenovirus encoding an inducible-active Akt was able to overcome the blockade of differentiation caused by IGFBP5 and restored normal osteogenesis. The team concluded that an intact IGF-induced PI3-kinase-Akt signaling cascade is essential for osteoblast differentiation

and maturation, bone development, and growth, and suggested that manipulation of this pathway could facilitate bone remodeling and fracture repair in athletes.⁷³

After reviewing genes related to the athletic performance and setting examples of some desired traits spanning strength, endurance, pain relief, and fracture repair, we agree that the sport genetics field is still in its early stage and more detailed gene maps will appear in the future with much higher resolution of the genetic elements. The list of related genes will expand and more polymorphisms will be identified. This will enhance our understanding of the athletic potential and shift athlete selection process toward an educated decision as compared to a semirandomized one.

7.3 MOLECULAR AND EPIGENETIC CHANGES DURING EXERCISE

It is well known that exercise enhances coordination, stress resistance, and stress coping capabilities; however, the details and locations of the short-term memory effects formed after exercise on the central genetic dogma are still ambiguous despite speculations and assumptions actively being made. Recently, a novel mechanism was suggested connecting epigenetic changes and gene transcription activities taking place at dentate gyrus neurons in response to training, forming the base for performance and stress response enhancement of exercise.⁷⁴ The study attributed the performance enhancement of exercise to increased histone H3 phosphorylation and increased c-Fos protein induction.

Histones are basic proteins that associate with DNA in a cell nucleus.⁷⁵ They are rich in basic amino acids (around 20% of their amino acids compositions are arginines and lysines).⁷⁶ Electrostatic interactions between the positive charges of these amino acids and the negative charges of phosphate groups in the DNA backbone mediate packaging of DNA into chromatin.⁷⁷ Two copies of each of the four core histones (H2A, H2B, H3, and H4) form an octamer. DNA (around 147 base pairs) is wrapped around this octamer to produce a nucleosomal core particle.⁷⁸ The second level of organizing chromatin comes with what is known as a linker region where 20–60 bp of DNA link one nucleosome to another. Each linker region is occupied by a single molecule of histone H1, giving a “beads on a string” appearance.⁷⁹ This 11-nm histone fiber is then further packed into an irregular 30-nm chromatin fiber structure that is coiled into even more complex structures to eventually assemble the chromosome (Figure 7.1). Binding of histones to DNA does not depend on a particular nucleotide sequences in the DNA but does depend critically on the amino acid sequence of the histone.⁸⁰ Histones are some of the most conserved proteins in eukaryotes. Calf histone H4 differs from pea H4 by only two amino acid residues.⁸¹ Binding of transcription factors to gene promoters may be inhibited if the promoter is blocked by a nucleosome, and is usually associated with sliding nucleosomes along the DNA molecule, exposing the gene’s promoter so that the transcription factors can access that region.^{82,83} Transcription of protein-coding genes is carried out by RNA polymerase II (RNAP II).⁷⁷ In order for the polymerase to travel along the DNA, a complex of proteins removes the nucleosomes in front of RNAP II and then replaces them after RNAP II has transcribed the sequence. This removal of histones in front of RNA polymerases and putting them back after transcription is completed is known as a chromatin-remodeling event.^{83,84} Two major types of chromatin exist

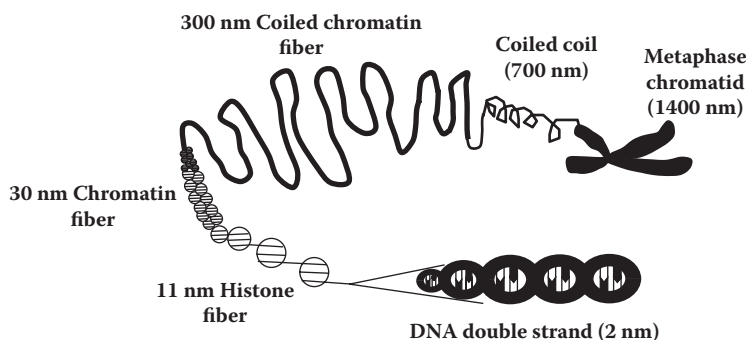


FIGURE 7.1 Chromatin structure.

in the cell nucleus: heterochromatin and euchromatin.^{85,86} Heterochromatin is the chromatin that is condensed during the interphase whereas euchromatin is actively transcribed.⁸⁷ Around 75–80% of the histone amino acids are incorporated in the core and only the N-terminal tails of histones protrude from the nucleosomal surface. Through modifications of the side chains of different amino acids on the N-terminus (exposed side of the nucleosomes) of histones, the chromatin structure can be controlled and part of the DNA sequences can be actively expressed or silenced.^{75,76,88} As stated above, chromatin remodeling is mediated through various chemical modifications of amino acid residues in histones; these modifications include covalent attachment of acetyl groups (CH_3CO^-) to lysines, phosphate groups to serines and threonines, methyl groups to lysines and arginines, biotin to lysine groups, ubiquitinylation and sumoylation of lysine residues, and poly-ADP-ribosylation of glutamic or aspartic acid residues.^{81,89–98} Chemical modifications occur on these tails, especially for H3 and H4 histones (Figure 7.2).^{86,99} The most important feature of these modifications is that they are reversible. For example, acetyl groups are added by enzymes called histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs).^{100,101} Acetylation of histone tails occurs in regions of chromatin that become active in gene transcription.^{102,103} Adding acetyl groups neutralizes the positive charges on lysines, thus reducing the strength of the association between the negatively charged DNA and the positively charged histones. Likewise, methylation, which also neutralizes the charge on lysines (and arginines), can either stimulate or inhibit gene transcription in that region.^{88,104,105} Methylation of lysine-4 in H3 is associated with active genes while methylation of lysine-9 in H3 is associated with inactive genes.^{106–108} It is now clear that histones are a dynamic component of chromatin and not simply inert DNA-packing material. All these modifications are part of what is known as the histone code.^{85,89,109,110}

Collins et al.⁷⁴ took a close look at how well-exercised lab rats perform better under stressful environments than their control (rested) counterparts. The team showed that epigenetic mechanisms in the brain played a major role in this adaptation. Rats were initially divided into two groups: The first experimental group was trained on an exercise wheel for 4 weeks, while the second group did not undergo any training or exercise regimen (control group). Both groups were then subjected to

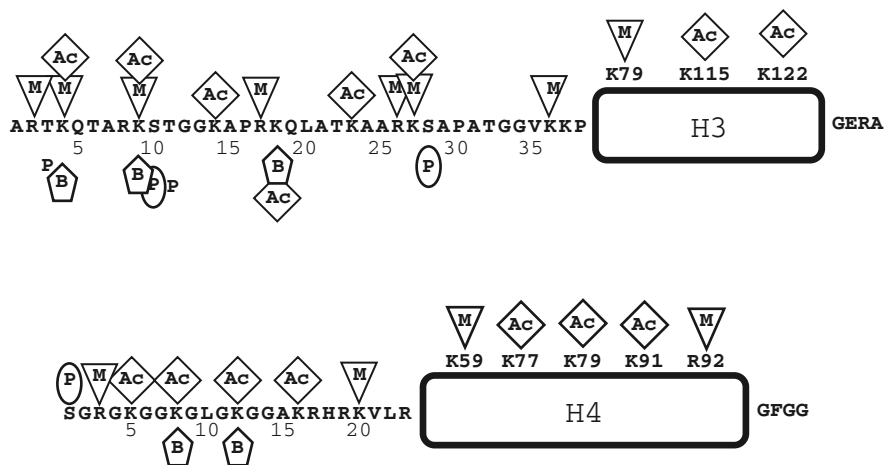


FIGURE 7.2 Modification sites in histones H3 and H4. Ac = Acetate; B = biotin; M = methyl; P = phosphate; U = ubiquitin.

two types of stresses: novel environment exposure and forced swimming. After tests were conducted, dentate gyrus tissues were collected and examined for histone H3 phospho-acetylation and c-Fos induction. During the novel environment exposure test, the unexercised group was more nervous, exploring their surroundings during the whole testing period, whereas the trained group was more relaxed and finished exploring the testing stage within 15 minutes of start time.

In the forced swimming experiment, the rats were placed in a container of water for 15 minutes and observed. This was repeated again 24 hours later for 5 minutes. Both exercised and control groups showed similar behavior in the initial test, but later in the repeat, the exercised rats showed better mobility, coordinated behavior, and less struggling as compared to control rats. It was concluded from these results that the exercised group was better able to cope and create memories of the first stressful event, enabling them to react better when exposed to the same stressful event the second time. When brains of these rats were harvested and examined using immunohistochemical techniques, the research team found significant increases in histone H3 phospho-acetylation and induction of c-Fos in the brains of the exercised rats. Others have reported similar observations that confirm this model connecting behavioral observations, epigenetic changes, enhanced stress response, and memory formation.^{111–113}

7.4 IMPLICATIONS FOR NUTRITIONAL ASSESSMENT

It has been known for decades that the nutrition requirements of athletes differ substantially from the rest of the human population. In order to repair muscles and excrete metabolic by-products, there are increased demands for energy, proteins, amino acids, and several metabolic cofactors such as vitamins and minerals. What is considered sufficient for an active person with 2000-calorie intake daily would

definitely be considered an inadequate intake for an Olympic swimmer who needs a 6000-calorie daily intake.

Despite the early knowledge of these higher demands of athletes, it was only recently elucidated that a connection exists between physical activity, genetic polymorphisms, and the elevated needs in those individuals. Murakami et al.¹¹⁴ were the first to connect genetic polymorphisms affecting the vitamin D receptor to low resistance training. These researchers reported that some SNP (single-nucleotide polymorphisms: changes in the genomic DNA sequence affecting only one single nucleotide) patterns show better improvement of parameters associated with the effects of low resistance training. In another study that was conducted on adolescent soccer players in Brazil, Diogenes et al. demonstrated that FokI polymorphism in vitamin D receptor (*VDR*) genes affected bone mass in those players and suggested that the FokI effect on bone mineralization occurs during bone maturation, possibly at the initial pubertal stages. This observation was confirmed by measuring total body bone mineral content (TBMC), total body bone mineral density (TBMD), insulin-like growth factor-I (IGF-I), testosterone, intact parathyroid hormone, and finally the activity of bone alkaline phosphatase found in the plasma.¹¹⁵ Certain polymorphisms not only have been reported to affect the athletic ability, but in fact some of these polymorphisms, such as the one that is found in intron 8 (ApaI) and exon 9 (TaqI) of vitamin D receptors, have long been known for their reverse health effect and association with increased risks of chronic disease development such as colorectal¹¹⁶ and renal¹¹⁷ cancers.

What is true for vitamin D is also true for several other vitamins and cofactors in increasing the risk of cancers and other diseases. These risks are documented and the relations between folate, vitamin B₆, and vitamin B₁₂ with breast cancers.^{118,119} as well as that between riboflavin, vitamin B₆, and vitamin B₁₂ with the risk of new colorectal adenomas,¹²⁰ are well established.

7.5 GENE TRANSFER AND POTENTIAL APPLICATIONS IN THE FIELD OF SPORTS NUTRITION

Molecular biology is one of the most rapidly growing scientific disciplines. This field is concerned with studying molecular structures and events underlying biological processes and understanding the relationship between genes and the cellular characteristics determined by these genes. The human genome contains around 50,000 to 100,000 genes, and each gene is responsible for the synthesis of a specific cellular protein/enzyme. Multiple forms of each protein might exist due to phenomena of mRNA alternative splicing and/or post-translational modification of proteins (included but not limited to glycosylation, phosphorylation).

Exercise physiologists pay close attention to cellular processes and the signals that trigger them, such as signals that regulate protein synthesis by turning on or turning off specific genes. Understanding the relationship between exercise and such factors is of invaluable practical importance. The recent technical revolution in the field of molecular biology offers opportunities to make use of scientific information for the improvement of human performance. For example, training results in modifications in the amounts and types of proteins synthesized in the exercised muscles. Indeed, it

is well known that regular strength training results in increased muscle size due to an increase in contractile proteins. Pointing out the exact molecular targets of such phenomenon might lead to more efficient training programs. In the following paragraphs, some of the technical issues related to the use of our accumulated knowledge of genomics and proteomics in the field of enhancing human athleticism are addressed.

In sports, there is an extended list of reasons why individuals might want to test their genomes/gene combinations. Among these reasons is the search for certain risk factors (weakness or increased susceptibility to injuries) or just the eagerness to confirm a potential athletic ability.

Recent advancements in biotechnological and molecular tools have resulted in the availability of commercial kits to test the presence/absence of certain genes and variants. Genes reported earlier in this chapter and involved in endurance, muscle growth, fracture repair, and pain tolerance are among the alluring targets for such detection kits. In fact, companies are in a fast race to produce and validate easy-to-use kits that could be easily used with little instrumentation to serve the sportsperson. The first commercially developed kit on the market targeted the *ACTN3* gene involved in regulating the fast-twitch muscle fiber function. With simple steps of collecting a biological sample (usually saliva) and a polymerase chain reaction (PCR) assisted amplification of the targeted coding region, the available allelic combination could be deciphered. Athletes with two disrupted copies (homozygous) of the *ACTN3* gene might be oriented to endurance sports rather than sports that require explosive power.²⁴

Gene testing is expected to expand in the future. These methods do not always give a reliable prediction, but individuals seeking to choose their own sport participation might find such testing very helpful. Sport coaches testing young team members to select professional careers, physicians predicting risks of illness and advising for preventative measures, and insurance companies seeking to estimate career-threatening injuries based partly on genetic information will heavily rely on these kits in the near future.

In this regard, it should be mentioned that other personal and social factors also contribute to the motivation of individuals to embrace certain types of sports. In reality these factors are as important as the athletic ability detected by genetic tests. In fact, genetic tests may turn problematic and start challenging our ethical code of conduct if these tests are used to form the only base for including/eliminating individuals from certain activities.

7.6 GENE “DOPING” AND ITS DETECTION

In many sports, such as track and field, it takes at least 8–10 years of hard training and intensive collaboration between coaches, nutritionists, and physicians to create high-caliber champions starting from talented trainees. With the “winner takes all” attitudes prevailing in sports, the fine line between success and failure, between fame and the attractions connected to it compared to going home with only silver/bronze medals, makes it very alluring to use any performance-enhancing method available on the market at the time of competition without paying much attention to the ethical consequences related to such use.^{1,2}

The recent advances of gene therapy methods could be effectively used to enhance athletic performance. The difficulty in distinguishing transgenic proteins from wild-type partners makes these approaches more attractive. Generally, any method that depends on the horizontal transfer of genes in order to enhance performance is designated as “gene doping.” According to the World Anti-Doping Agency, gene doping is defined as “the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance.”¹ Gene doping not only undermines principles of fair play in sports, but most importantly it involves major health risks to athletes who partake in gene doping and it forms a real threat to the world of sports in human populations.^{121–124}

From a technical point of view, there are two approaches for using genetic engineering in enhancing performance. The first approach depends on expressing and purifying engineered proteins/enzymes in preferred hosts such as *E. coli*, *Saccharomyces cerevisiae*, and human cells. The purified and tested proteins will be then injected for their effects. This approach has some disadvantages such as triggering strong immune reactions leading to inflammation, low expression levels achieved with this method, and finally the need to repeat the injection every time the accompanied effect is desired.^{1,125} The second approach depends on introducing viral particles carrying transgenic proteins/enzymes to integrate within the infected cell’s genome. These vectors can be inducible/noninducible depending on their nature, and they carry some additional regulatory genetic elements. Expression of inducible vectors can be under the control of certain chemicals (such as antibiotics like tetracycline or doxycycline) to start locally producing the transgenic protein/enzyme. These chemicals can help in regulating the timing and duration of gene expression. Tissue-specific promoters could be integrated within the vector backbone so the gene expression takes place only in specific tissues. The second approach eliminates any possible allergenic reactions toward the engineered protein/enzyme.^{1,126,127}

The gene therapy that was originally designed to overcome serious human diseases is now a major challenge facing our definition of fair competition in today’s sport. In less than 30 years of development, gene therapy is the predominant topic that covers sports ethics and sportsmanship. In addition to the ethical side of this issue, the danger of spreading viral particles carrying vectors encoding proteins or performance enhancers in humans is not appealing at all.¹²⁸

Since the early realization of the threat of gene doping in sports, scientists from different disciplines have been concerned with potential misuses of gene therapy technologies and have invested extensive efforts to develop robust methods for gene-doping detection. Different technologies are being optimized for the detection of gene doping. Generally speaking, factors such as detection target, type of sample required for analysis, and the response of the body at both cellular and systemic levels should be considered when evaluating strategies for gene-doping detection. The current available knowledge of many fields such as gene technology, immunology, transcriptomics, proteomics, biochemistry, and physiology is being utilized in order to establish reliable detection methods. So far, only protein biomarkers are proving to be successful as indirect indicators of gene doping.^{129,130} In principal, transgenic proteins once introduced into the cell may alter the proteomic content of that cell and affect the metabolic pathways within that cell. In addition, they might produce some

characteristic immune responses (as they are initially carried by viruses). Tracking changes in the proteome, metabolic pathways, and/or immunological responses might prove to be the only way to discover gene doping.^{129,131}

7.7 CONCLUSIONS

Every individual comes with a genetic map that dictates some of his or her future potentials in every aspect of life. Genes might mark the sidelines of life's highway, but certainly the genes do not have the power to choose which lane we take. Factors such as nutrition, training programs, family support, education, motivation, culture, and social background complement the genetic map to define us as human beings. Having certain genes that give us the advantage over others in sport and physical performance is desirable, but this gift should be nourished with training and eagerness to excel in order to achieve our maximum potentials.

While gene therapy and its possible misuse in sports doping is still in its infancy, the time will come soon when it will form a major challenge for sportsmanship. The commercialization of every aspect of our lives is an alluring and driving force for sport personnel to win gold medals, break world records, and hence sign big contracts for advertising companies. Resources should be invested in developing analytical methods for tracking changes in cellular pathways to increase our chances of detecting gene doping and protect human populations from uncontrolled and misused gene technology.

Fair competition principles and ethical practices should be included in the education of people working in sports. Setting examples of successful fairly competing champions, in addition to pointing out misconducts and their severe consequences, should be part of this process.

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Section IV

Biochemical Assessment of Athletes

8 Assessment of Lipid Status of Athletes

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8.1 INTRODUCTION

The incidence of obesity and metabolic syndrome in the United States has reached an epidemic level and has increased in prevalence even among our nation's youth.¹ In addition, athletes in some sports like American football and basketball are larger than ever and would be considered obese by conventional body mass index (BMI) and/or percent body fat criteria.² A recent study examining the prevalence of markers of metabolic syndrome in 70 Division I, II, and III American football players found a disturbing incidence of athletes with abdominal adiposity, high percent body fat, and low high-density lipoproteins (HDL).³ In addition, they had high blood pressure, fasting blood glucose, fasting cholesterol, and serum triglycerides.³ In terms of blood lipids, the researchers found that 46% of the athletes had total cholesterol to HDL ratios greater than 5.0, 17% of the athletes had total cholesterol above 200 mg/dL, and

24% of the athletes had low-density lipoproteins greater than 130 mg/dL. Further, 21% of the athletes had elevated C-reactive protein values (an indicator of inflammation) while 49% were found to have clinically diagnosed metabolic syndrome. The presence of several of these risk factors significantly correlated to abdominal obesity and body fat percentage. The researchers recommended that all athletes who had an abdominal circumference of greater than 100 cm (40 inches) be screened for risk factors associated with metabolic syndrome. While many presume that athletes are lean, fit, and have low blood lipids, given the incidence of hyperlipidemia and dyslipidemia in the population, this assumption may be misguided. Consequently, the sports medicine professional must understand the proper methods of assessing and managing lipid status of athletes. This chapter presents an overview of general lipid metabolism, lipid screening processes, and ways to manage athletes found to have high blood lipids.

8.2 GENERAL LIPID METABOLISM

Studies of dietary intake of athletes have shown their macronutrient composition to be similar to those of the standard U.S. population, differing only in the amount of calories consumed.⁴ Therefore, for practitioners involved directly in the performance of athletes, it is necessary to understand the components of their diet to make clear suggestions and educate them on their dietary needs. The purpose of this section is to highlight the importance of lipids by providing an explanation of lipid metabolism, both exogenous and endogenous, as well as to provide guidelines for assessing lipid profiles in athletes for the determination of future health risk.

Lipids consist of triglycerides, phospholipids, and cholesterol, and serve as a major biological component of cells as well as a fuel source for energy for many human processes. The majority of dietary lipids come in the form of triglycerides, while dietary cholesterol contributes to a much smaller degree.^{5,6} The metabolism of lipids involves several processes to enable their use for biological components and energy, including digestion and absorption, transport of dietary lipids, endogenous lipid production, and reverse cholesterol transport.

8.2.1 DIETARY LIPIDS

Dietary fats consist of triglycerides, phospholipids, and cholesterol. They are classified by the chemical structure based on the number of fatty acids and carbons they contain. Medium-chain fatty acids are considered shorter than 10 carbons in length, while long-chain fatty acids are 10–12 carbons or longer in length. Fatty acids can be further classified by their chemical structure into four categories (saturated, monounsaturated, polyunsaturated, and transaturated fats). Saturated fatty acids contain the maximum number of hydrogens attached to each carbon in the chain. Saturated fats are usually solid at room temperature and come almost exclusively from animal sources. Overconsumption of this type of fat has been reported to be a risk for cardiovascular disease.

Monounsaturated and polyunsaturated fats are missing at least one pair of hydrogen atoms attached to a carbon and have a double bond in its place. Monounsaturated

fats only have one double bond, while polyunsaturated fats have more than one double bond. When the double bonds of monounsaturated or polyunsaturated fatty acids exist on opposite sides, these fatty acids are referred to as trans fatty acids. This is usually the result of hydrogenation, the artificial adding of hydrogen bonds, which is used to increase shelf life of many products. Monounsaturated fats can be found in oils such as olive, canola, and peanut. In addition, salmon, mackerel, halibut, trout, and shellfish are also high in monounsaturated fats. Polyunsaturated fats can be found in foods such as fish oils; seafood; polyunsaturated margarines; vegetable oils such as safflower, sunflower, corn, or soy oils; nuts such as walnuts and brazil nuts; and seeds. Unsaturated fatty acids also contain a special subgroup known as essential fatty acids, due to the body's inability to make these fatty acids in the body. Therefore, these fatty acids must be obtained in the diet and include omega-3 and omega-6 unsaturated fatty acids. "Omega-3" and "omega-6" describe where the double bonds exist in the fatty acid, counting from the terminal end of the fatty acid.^{7,8} A number of health benefits of a diet high in unsaturated fats and essential fatty acids have been reported. Consequently, it is generally recommended that total fat intake be limited to 30% or less of total energy intake, with the majority of dietary fats consumed in the form of unsaturated fats.

8.2.2 DIGESTION AND ABSORPTION

The process of digestion of dietary lipids begins in the stomach. Lingual lipase, increased by neural stimulation and by the intake of dietary fat, is secreted by the salivary glands.^{6,9} As lingual lipase combines with foodstuff in the mouth and moves into the stomach, the sheer stress caused by passage of food through sphincters and the contractions of the stomach create an emulsion, allowing lingual lipase to come in contact with the ingested lipids.⁶ The acidic pH of the stomach is ideal for the lingual lipase to facilitate the enzyme's ability to break down the lipid contents. Once the stomach has mixed all its contents, the partially digested lipid emulsion moves into the small intestine where the majority of digestion of lipids occurs.

Upon ingestion of a lipid-rich meal, the gallbladder contracts in response to cholecystokinin (CCK) secreted from the small intestine. The contents of the gallbladder, which include bile acids and lecithin produced in the liver, is secreted into the small intestine, where with the newly formed emulsion creates a favorable pH for lipid digestion.^{6,10,11} While bile acids contain no enzymes to break down lipids, the process of emulsification is essential in lipid digestion. The bile salts contained within the bile acid work as a detergent with contractions of the small intestine to break up the lipid emulsion. Enzymes secreted by the small intestine also work to further break down the lipids. CCK stimulates the release of pancreatic lipase, phospholipase A-2, and cholesterol esterase. Pancreatic lipase breaks down triglycerides into free fatty acids, diglycerides, and monoglycerides. Phospholipase A-2 works on phospholipids resulting in the production of a monoglyceride and lysophospholipid. The final enzyme in the process is cholesterol esterase, which metabolizes cholesterol into free cholesterol and a monoglyceride.⁶ After this breakdown has occurred, the fatty acids are absorbed by the intestinal mucosal cells.

At this point in the process, approximately 98% of the ingested triglycerides, along with 15–40 g of endogenous lipids, have been absorbed⁶ and are ready for transport. The small intestine is very efficient in its absorption of triglycerides, resulting in very little triglyceride in fecal excrement. However, only about 30% of dietary cholesterol has been absorbed at this point.¹² Cholesterol in the form of bile appears to be better absorbed than its dietary counterpart. This is partly due to the higher secretion rate of biliary cholesterol, which is two times the amount of dietary cholesterol, as well as the physical composition of cholesterol in bile. Dietary cholesterol entering into the small intestine requires emulsification by the bile salts and lecithin into the micellar state, whereas biliary cholesterol enters the intestine in the micellar state.¹² This could contribute to the less-than-optimal absorption of dietary cholesterol. Endogenous cholesterol production accounts for this deficit and increases when dietary consumption is low and decreases when dietary cholesterol is high.¹²

8.2.3 LIPID TRANSPORT

Small- and medium-chain triglycerides (10 to 12 carbons in length) can enter directly into the blood for transport after absorption by intestinal cells. This is due to their reduced hydrophobic nature compared to that of longer-chain fatty acids. Larger-chain fatty acids (greater than 12 carbons in length) are more hydrophobic and require further packaging. The ability of these lipids to be transported in plasma depends on their incorporation into lipoproteins. Lipoproteins contain lipids and transport proteins termed apoproteins (apo). Lipids and their apoprotein counterparts are termed apolipoproteins. Apolipoproteins are classified by their size, lipid content, apoprotein content, and subfraction of major lipoprotein class. Apoproteins are listed in Table 8.1 with their associated lipoprotein and their major functions.

TABLE 8.1
Types of Apolipoproteins, Associated Lipoproteins, and Major Functions

Apolipoprotein	Associated Lipoproteins	Major Functions
A-I	Chylomicron, HDL	Cholesterol acceptor from peripheral cells through ABCA1; cofactor for LCAT; facilitates lipid uptake
A-II	HDL	Displaces apo A-I; facilitates lipid uptake
B-48	Chylomicron, HDL	Assembly and secretion of chylomicrons from small intestine; structural component
B-100	VLDL, IDL, LDL	Assembly and secretion of VLDL from liver; LDL receptor
C-I	Chylomicron, VLDL, HDL	Inhibits hepatic uptake of chylomicron and VLDL remnants
C-II	Chylomicron, VLDL, HDL	Cofactor for LPL
C-III	Chylomicron, VLDL, HDL	Inhibits LPL
apo E	Chylomicron, VLDL, HDL	Facilitates lipid uptake through LDL receptor
apo(a)	Lp(a)	Most likely inhibits fibrinolysis

The four major classes of lipoproteins are chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Low-density lipoproteins are often classified further by their density into intermediate-density lipoproteins (IDL) and at the lower end of the density spectrum, LDL.¹³

Once absorbed in the intestinal mucosal cells, longer-chain fatty acids are packaged into chylomicrons to facilitate transport. Apo B-48 is essential in the transport of exogenous lipids in chylomicrons from the intestine. Upon secretion from the mucosal cells of the intestine, the chylomicrons enter the lymphatic system where several changes occur, including the addition of several apoproteins that allow the lipoprotein to be metabolized upon entering the bloodstream. The most important apoprotein addition is that of apo CII. Apo CII serves as a cofactor for lipoprotein lipase (LPL), which is synthesized in adipose and muscle cells.¹⁴ LPL catalyzes the hydrolysis of the triglyceride from the chylomicron, allowing the fatty acid to be taken up by adjacent tissues (mostly muscle and adipose) to be stored or used as energy.¹⁵ What remain after the hydrolysis are small particles of the chylomicron, which include cholesterol, phospholipids, apolipoproteins, and very little triglyceride. These remaining particles can then be integrated into HDL, which occurs with the majority of apo A and some apo C, with the remaining being catabolized by the liver.^{13,14,16–18} Figure 8.1 diagrams the process of lipid transport and endogenous production and transport.

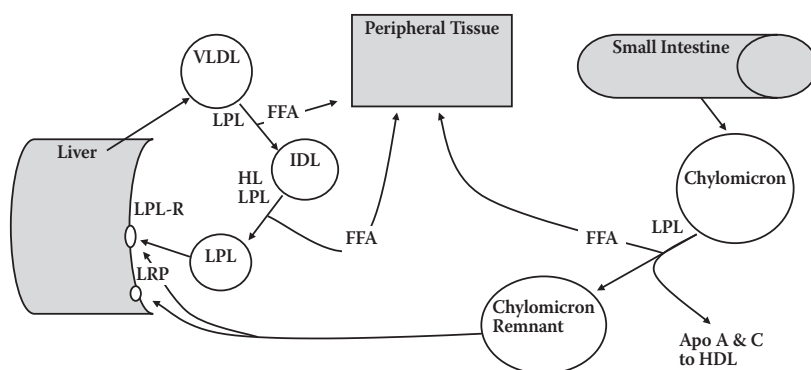


FIGURE 8.1 Lipid transport and endogenous lipid production. Once absorbed in intestine, large-chain fatty acids are secreted in chylomicrons which enter the lymphatic system where apolipoproteins are added to aid in transport. When the chylomicrons enter the blood, LPL catalyzes the hydrolysis releasing the fatty acid (FFA) to the peripheral tissues, while the apo A & C are integrated into HDL while the remaining chylomicron remnant is taken up and disposed of by the liver. Lipids produced by the liver are released into circulation in VLDL. LPL hydrolyzes the triglyceride in VLDL releasing the FFA to the peripheral tissues. This reduces the VLDL to IDL which is again hydrolyzed by LPL and HL (hepatic lipase) releasing the remaining FFA to peripheral tissues allowing the final LDL product to be taken up by the liver to be made available for other processes. (Adapted from Davis, P.G. and Waggoner, J.D., in *Lipid Metabolism and Health*, Moffatt, R.J. and Stamford, B., Eds., CRC Press, Boca Raton, FL, 2006, pp. 47–60.)

8.2.4 ENDOGENOUS PRODUCTION

Although the liver prefers the use of dietary lipids and those stored in adipose tissue, it also has the ability to produce lipids, which becomes important during the fasting state. Lipids produced by the liver (endogenous pathway) are released in VLDL, which utilizes a number of apoproteins. Of importance are apo B-100, a longer version of apo B-48, and apo CII, which serves again as the cofactor for LPL. In the case of endogenous lipids, LPL hydrolyzes the majority of the triglyceride contained within VLDL. Once hydrolyzed they become less dense and are converted to IDL and further into LDL. The remaining LDL particle is taken up by liver and adrenal cells to be made available for membrane structures and steroid hormone synthesis.^{13,14,16–18}

8.2.5 REVERSE CHOLESTEROL TRANSPORT

The body has yet another mechanism that provides a cardioprotective benefit in the removal of cholesterol from peripheral tissues and vascular lesions. This mechanism utilizes HDL, which works in conjunction with adenosine triphosphate-binding cassette-A-I (ABCA-I) transporter. Briefly stated, the small apo A-I of HDL is formed in the lymph and delivered into the circulation where it interacts with peripheral tissues and other areas in the arterial walls that contain free cholesterol. Apo A-I removes free cholesterol with assistance from ABCA-I. The free cholesterol then incorporates into the apo A-I HDL. As more cholesterol is incorporated, it becomes a strong cofactor for lecithin-cholesterol acyltransferase (LCAT). This allows further packaging of the cholesterol within the HDL, which through interaction with LCAT becomes HDL2. The Sr-B1 receptor on the liver recognizes the mature HDL and allows for crossing into the liver, resulting in its excretion in bile.¹⁸

8.3 ATHLETE SCREENING

Many studies have evaluated the lipid profiles of athletes. Most studies indicate that athletes have lipid profiles that are associated with a lower incidence of cardiovascular disease (CVD).¹⁹ The most beneficial effects seen in most of these studies has been the increased levels of HDL, particularly in endurance athletes.¹⁹ A reduction of triglycerides and an increase in HDL appear to be long-term effects of exercise training.²⁰ However, athletes are not immune to lipid disorders, including dyslipidemia, and should therefore be screened appropriately. This is increasingly true among college athletes; few studies have studied this age group, as little data exists as to the prevalence among this age group. In a recent study of collegiate athletes, 10% reported having cholesterol levels greater than the current recommendation levels, while 24% of males and 30% females had HDL levels lower than recommended values.²¹ The majority of studies on athletes have focused on the beneficial effects experienced by endurance athletes; however, while strength and power athletes experience some protective benefits of training, their profiles differ overall. This has been shown to be true in athletes with a higher BMI,²² such as football linemen, and those participating in little endurance training, such as power lifters

and throwers.^{23,24} Currently, a different means of analyzing lipid profiles of athletes compared to their sedentary counterparts does not exist. For this reason, the standard for the normal population is discussed, with emphasis placed on where athletes may differ.

8.3.1 TYPES OF LIPID DISORDERS

There are several types of dyslipidemia disorders that practitioners should be aware of if they are involved in screening of hyperlipidemia. The Fredrickson classification system has been developed and adopted by the World Health Organization to characterize different types of hyperlipoproteinemia disorders.²⁵

Hyperlipoproteinemia Type I is a rare condition associated with deficiencies in the enzyme lipoprotein lipase (LPL) or its cofactor apolipoprotein CII. The LPL enzyme is found primarily in endothelial cells and serves to hydrolyze lipids found in chylomicrons and VLDL into free fatty acids and glycerol. Individuals with Type I hyperlipoproteinemia typically have high cholesterol, marked elevations in chylomicron levels, and triglyceride levels ranging from 1,000 to 10,000 mg/dL. It is usually treated with diet control and is not associated with elevations in risk to cardiovascular diseases.

Hyperlipoproteinemia Type II is the most common dyslipidemia and is further classified as Type IIa or Type IIb hyperlipoproteinemia.

Type IIa hyperproteinemia is also known as polygenic hypercholesterolemia of familial hypercholesterolemia. This is a genetic-related disorder associated with LDL receptor deficiency. Individuals with Type IIa hyperlipoproteinemia present with elevated cholesterol, LDL, and VLDL. Individuals with Type IIa hyperproteinemia also may have tendon xanthomas (deposition of yellowish cholesterol-rich material in tendons) and/or xanthelasma (deposition of yellowish cholesterol-rich material on eyelids). It is usually treated with bile acid sequestrants, statins, and niacin therapy.

Type IIb hyperproteinemia (also known as combined hyperlipidemia) is similar to Type IIa hyperproteinemia with the exception that this form of dyslipidemia is also associated with elevated triglyceride levels. Individuals with Type IIb hyperproteinemia typically have elevated cholesterol, LDL, and triglycerides typically due to an inability to metabolize and/or clear fats in the liver. It also may be associated with low HDL cholesterol. They also have an increased incidence of metabolic syndrome. Patients with Type IIb hyperlipoproteinemia are typically treated with diet therapy, fibrate medications (which work on peroxisome proliferator-activated receptors to decrease free fatty acid production), and statin drugs (which can reduce LDL levels by promoting LDL uptake in the liver by increasing LDL-receptor expression). Both of these forms of dyslipidemias are associated with an increased incidence of cardiovascular disease.

Type III hyperlipoproteinemia is also known as broad beta disease or dysbeta-lipoproteinemia. Type III hyperlipoproteinemia is a rare, genetically related dyslipidemia that affects about 0.02% of the population. Individuals with Type III hyperlipoproteinemia have the ApoE E2/E2 genotype. It is associated with elevations in cholesterol, triglycerides, chylomicrons, intermediate density lipoproteins (IDL), and risk of cardiovascular disease. Treatment typically involves diet therapy, fibrates, and statins.

Type IV hyperlipoproteinemia is a dyslipidemia disorder affecting about 16% of the population according to the NCEP-ATPIII criteria, where hypertriglyceridemia is greater than 200 mg/dL. Individuals with Type IV hyperlipoproteinemia have elevated VLDL production and a reduced capacity to eliminate VLDL. This dyslipidemia is characterized with elevations in triglycerides (200–1,000 mg/dL) and VLDL. However, total cholesterol levels are typically within normal ranges. Treatment options include diet therapy, fibrates, niacin, and statins.

Type V hyperlipoproteinemia is a rare condition that is commonly known as endogenous hypertriglyceremia. It is similar to Type I but is associated with high VLDL in addition to elevated cholesterol, chylomicrons, and triglycerides usually greater than 1000 mg/dL. Individuals who have Type V hyperlipoproteinemia typically have an increased production of VLDL, decreased LPL, glucose, glucose intolerance, and hyperuricemia. Treatment typically includes diet, niacin, and/or fibrate therapy.

8.3.2 LIPID MARKERS AND NORMS

The primary purpose for cholesterol screening is the determination of risk of development of CVD. The National Cholesterol Education Program (NCEP) recommends a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) for all adults aged 20 years and older every 5 years. In addition to a fasting lipoprotein profile, the NCEP also recommends an assessment of accompanying risk factors, which include cigarette smoking, hypertension (blood pressure \geq 140/90 mm Hg or on an antihypertensive medication), family history of premature cardiovascular disease, and age (men \geq 45; women \geq 55).²⁶ [Table 8.2](#) outlines the current guidelines provided by the NCEP for identification of risk. [Figure 8.2](#) provides the appropriate testing timetable with current recommendations.

Although cholesterol screening provides an assessment of CVD, the role of triglycerides in lipid abnormalities cannot be overlooked. Cross-sectional studies have shown that mean triglyceride levels have increased, particularly in the United States. Although HDL attenuates some of the risk associated with high triglycerides and the development of CVD, a full lipid profile and clinical assessment is needed to determine full risk. As recent data have shown, CVD risk is still apparent when patients present with low LDL.²⁷

It is important to note several factors play a role in lipids and lipoproteins. Age, weight loss, the use of anabolic steroids, and lifestyle choices such as smoking and alcohol consumption all contribute to the levels of circulating lipids. Cholesterol levels have been shown to increase with age, with a greater increase in men.²⁸ During

TABLE 8.2
NCEP Classification of LDL Cholesterol, HDL Cholesterol, Total Cholesterol, and Triglycerides

LDL Cholesterol (mg/dL)	
< 100	Optimal
100–129	Near or above optimal
130–159	Borderline high
160–189	High
≥ 190	Very high
HDL Cholesterol (mg/dL)	
< 40	Low
≥ 60	High
Total Cholesterol (mg/dL)	
< 200	Desirable
200–239	Borderline high
≥ 240	High
Triglycerides (mg/dL)	
< 150	Normal
150–199	Borderline high
200–499	High
≥ 500	Very high

Source: Panel E, Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III), *JAMA*, 285, 2486–97, 2001.

- Fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides)
 - If non-fasting, only total cholesterol and HDL are usable. A second fasting profile should be obtained if total cholesterol > 200 mg/dL or HDL < 40 mg/dL. If values are abnormal, a second profile should be obtained.
- Screening recommended for all adults aged 20 years or older every 5 years
- Other major risk factors
 - Smoking
 - High blood pressure (≥ 140/90 mm/Hg)
 - Low HDL (< 40 mg/dL)
 - Family history of cardiovascular disease
 - Age (men ≥ 45, women ≥ 55)
- Treatment decision should involve athlete and practitioner including dietary and pharmacological interventions.

FIGURE 8.2 Appropriate timetable for lipoprotein profile testing.

dieting and weight loss, the activity of LPL is altered, which can result in changes in the blood lipid profiles, and thus a lipoprotein profile should be conducted when the athlete is not trying to lose weight or restricting calories.²⁹ Anabolic steroid use can affect screening by altering lipoprotein fractions and increasing triglyceride levels.³⁰ Smoking, a risk factor acknowledged by the NCEP, has been shown to decrease HDL, while alcohol consumption has been shown to increase triglyceride levels.³¹

8.3.3 SCREENING PROCESS

In their most recent report, the NCEP established guidelines for the identification and treatment of persons with high cholesterol.²⁶ Based on these recommendations, athletes should be screened appropriately following these guidelines, since any benefits seen from training will be elucidated in the identification and evaluation process.¹⁹ Athletes who may be particularly susceptible are those with waist circumferences greater than 40 inches and/or a body fat percentage greater than 25%.³

The initial step set forth by the NCEP is the assessment of the person's risk. Risk assessment, as previously stated, should include a fasting lipid profile, which includes categorization based on LDL, HDL, and total cholesterol values listed in [Table 8.2](#). A fasting blood lipid profile (8–12 hours fasting) is recommended, as the concentration of blood lipids in blood can range from 5% to 10% daily. This variation can be attributed to several factors, including dietary intake, exercise, and alcohol consumption, to name a few. The blood is usually drawn from the antecubital space. Serum is typically used for determination of lipid profiles, and this requires an amount of blood that is greater than can be obtained from a finger stick.

For estimating cholesterol and triglyceride concentrations, enzymatic procedures are most commonly employed in the clinical setting. The enzymes required for these assay procedures may be prepared by the researcher, but most are commercially available, which makes them the most widely used clinically. Determination of cholesterol involves three enzymes: cholesterol esterase, cholesterol oxidase, and peroxidase. Cholesterol esterase hydrolyzes the cholesterol esters to free cholesterol and fatty acids. Cholesterol oxidase then oxidizes the free cholesterol. The final step is a quinoneimine dye, which is produced when hydrogen peroxide oxidizes *p*-hydroxybenzenesulfonate and 4-aminoantipyrine in the presence of the peroxidase.^{32–34} Cholesterol can then be indirectly quantified using spectrophotometry. HDL can be measured by precipitating non-HDL cholesterol with heparin and measuring the total cholesterol remaining. Currently, in clinical setting the most common procedure used to determine LDL is using the Friedewald calculation. This calculation utilizes total cholesterol and HDL to estimate LDL. This practice, however, suffers from several limitations.^{35,36} Other more accurate methods for determination of HDL and LDL have been developed using their density and/or charge. These methods employ the use of ultracentrifugation and electrophoresis.^{36,37}

Triglyceride determination involves a different set of four enzymes: lipoprotein lipase, glycerol kinase, glycerol phosphate kinase, and peroxidase. Lipoprotein lipase serves to hydrolyze the triglycerides into fatty acids and glycerol. Glycerol from the first step can then be phosphorylated to glycerol-1-phosphate and adenosine-5-diphosphate. Glycerol phosphate kinase can then oxidize the glycerol-

1-phosphate to dihydroxyacetone phosphate and hydrogen peroxide. Again, a quino-neimine dye is formed when hydrogen peroxide reacts with 4-amino-antipyrine and 5-dichloro-2-hydroxybenzene sulfonate. Triglyceride concentration can be indirectly quantified using spectrophotometry.^{32–34}

In addition to a fasting lipid profile, the presence of clinical coronary heart disease (CHD) should be determined. The next step in risk assessment is determining the presence of the other major risks: cigarette smoking, hypertension (blood pressure ≥ 140/90 mm Hg or on an antihypertensive medication), family history of premature cardiovascular disease, and age (men ≥ 45; women ≥ 55).²⁶

After determination of risk, clinicians can then divide the athlete into one of three categories based on the presence of CHD and other risk factors, with the appropriate goal for LDL. If the athlete has the presence of CHD and any risk factors, his or her goal LDL is < 100 mg/dL. If he or she has more than two risk factors and no presence of CHD, then the goal LDL is < 130 mg/dL. The lowest risk, those with 0–1 risk factors, should have an LDL profile of < 160 mg/dL. When more than two risk factors are present, the clinician should do a 10-year risk assessment carried out using the Framingham scoring to determine if intensive treatment is necessary.^{19,26}

Table 8.3 outlines the goal LDL levels for the three groups based on risk assessment and the corresponding point at which to begin therapeutic lifestyle changes (TLC), as defined by NCEP, or drug therapy. Athletes are a special population and thus require added attention at this point; any therapy, whether it be TLC or drug related, must also focus on ensuring the athlete can still participate in his or her sport.¹⁹ TLC, as recommended by the NCEP, includes reducing intakes of saturated fats to < 7% of total calories and cholesterol intake to < 200 mg/day, increasing physical activity, implementing weight reduction, inclusion of plant stanols/sterols in the diet, and increasing fiber intake.²⁶ Drug treatment is recommended after

TABLE 8.3
LDL Cholesterol Goals and Guidelines to Begin Therapeutic Life Changes and Drug Therapy

Risk Category	LDL Goal (mg/dL)	LDL to Start Therapeutic Lifestyle Changes (mg/dL)	LDL Level to Consider Drug Therapy (mg/dL)
CHD or CHD risk equivalents (10 yr risk > 20%)	< 100	≥ 100	≥ 130 (100–129, drug optional)
2+ risk factors (10 yr risk ≤ 20%)	< 130	≥ 130	≥ 130, 10 yr risk 10–20% ≥ 160, 10 yr risk < 10%
0–1 risk factor	< 160	≥ 160	≥ 190 (160–189, drug optional)

Source: Panel E, Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III), *JAMA*, 285, 2486–97, 2001.

three months of TLC if goal LDL levels are not met and should be evaluated and administered by a registered physician.¹⁹

8.3.4 OTHER METHODS OF DETERMINATION

More recent advances in the study of lipids and risk factors associated with the development of CVD have led to the discovery of other methods for the determination of risk. The NCEP currently only recommends the above guidelines in determining CVD risk, even though other methods discussed have shown to be a stronger predictor of the development of CVD. The reasoning for acknowledgment of only the NCEP guidelines is multifaceted. Some of the methods discussed have been shown to have no greater significant diagnostic value than the standard lipid profile.³⁸ For methods with greater significant value, the question remains as to how long a reeducation of physicians and patients alike would take and to what benefit these measurements could improve determination of CVD risk in those already at risk.^{39,40} Additionally, the tests required with some of these methods are not part of the standard lipid profile. Insurance carriers currently believe these to be experimental and thus the burden of cost would fall to the patient.⁴⁰ Therefore, the methods described only provide insight into the new ways of analyzing risk factors associated with the development of CVD.

Two ratios have been shown to be strong predictors of the development of CVD, the LDL-C/HDL-C ratio and the apo B/apo A-I ratio. The LDL-C/HDL-C ratio has been identified as associated with risk of development of CVD in several large-scale studies.^{40–42} However, the majority of these studies were conducted on middle-aged men, and this should be taken into account when assessing athletes. The level of apo B and the ratio of lipoproteins apo B/apo A-I have recently proved to be the most accurate predictors of CVD.^{43,44} The apo B is a structural protein responsible for VLDL transport of lipids from the intestine and the liver to tissues, while apo A-I is used in reverse cholesterol transport carrying particles to the liver for excretion.⁴³ The relationship of these two lipoproteins therefore provides a direct ratio of atherogenic to nonatherogenic.

C-reactive protein (CRP), an inflammatory marker, has shown to be strongly associated with the development of CVD. Studies have shown that CRP levels increase with age and are higher in the obese and smokers. Due to these factors, there has been a strong association between CRP and the development of CVD.⁴⁵ However, more recent studies have shown that CRP levels provide no greater diagnostic value than the standard lipid profile recommended by the NCEP.³⁸

8.4 LOWERING BLOOD LIPIDS

The NCEP has recommended using LDL levels in combination with assessing risk factors of CHD as a criterion in determining treatment options for patients with dyslipidemia. The greater the risk profile, the lower the LDL level needs to be to initiate treatment options. For individuals with moderately elevated LDL levels and a low risk-factor profile, lifestyle alterations involving increasing physical activity, losing weight, and consuming a low-fat and low-cholesterol diet are typically the

first treatment course.⁴⁶ Table 8.4 has dietary recommendations provided by NCEP as well as others for a healthy lifestyle. Those with more risk factors may require additional dietary and/or pharmacological interventions.

Sustained endurance or aerobic-based exercise (walking, running, cycling, swimming, etc.) have been reported to lower total cholesterol and LDL cholesterol while increasing HDL cholesterol.⁴⁷ In addition, increasing the amount of endurance exercise appears to have a dose-related benefit, particularly in individuals who have lower BMIs.⁴⁷ Individuals who primarily engage in high-intensity intermittent exercise or power-based physical activity (such as resistance training, American football, baseball, and softball) may not observe as much benefit of exercise training on blood lipid profiles unless they include some aerobic-based exercise in their training.⁴⁷ This is particularly true for larger athletes who have a high percent body fat or higher BMIs.³ Weight loss that may occur as a result of exercise training and/or caloric restriction has also been shown to have positive benefits on blood lipid profiles.^{48,49}

The second general guideline to help individuals lower blood lipids is to consume a low-fat and low-cholesterol diet. Animal sources of food (such as meats, poultry, shellfish, eggs, butter, cheese, whole or 2% milk) are relatively high in fat, saturated fats, and cholesterol. The American Heart Association (AHA) recommends that individuals consume 25–35% of their total daily caloric intake in the form of fat. In addition, the AHA recommends that saturated fat intake should be less than 7% of fat intake, transfat intake should be less than 1% of total daily caloric intake, and cholesterol intake should be less than 300 mg/day.⁴⁶ Moreover, individuals who are prescribed lipid-lowering medications should consume less than 200 mg/day of dietary cholesterol.

TABLE 8.4
Dietary Guidelines to Lower Blood Lipids and Composition
of NCEP TLC Diet

Saturated fat	Less than 7% per day
Polyunsaturated fat	Up to 10% total calories
Monounsaturated fat	Up to 20% total calories
Total fat	25–35% total calories
Carbohydrate	50–60% total calories
Fiber	20–30 grams/day
Protein	15% total calories
Dietary cholesterol	Less than 200 mg/day
Soluble fiber	5–10 grams/day
Plant sterols/stanols	3 grams/day
Omega-3-containing fish	2 × per week

Source: Panel E, Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III), *JAMA*, 285, 2486–97, 2001.

Additional dietary and/or pharmacological interventions may be needed for individuals at high risk of CHD with elevated LDL levels.⁴⁶ There are several nutritional strategies that may help in the management of dyslipidemia. For example, nicotinic acid (niacin) supplementation (up to 2 grams/day) has been reported to lower LDL, triglycerides, and lipoprotein-a cholesterol by 15–35% while increasing HDL cholesterol by as much as 25%.^{48,49} Although ingesting large amounts of niacin can cause flushing of blood to the skin, skin rashes, gastrointestinal distress, increases in uric acid levels, elevations in blood glucose, and liver dysfunction, longer-acting or slow-releasing forms of niacin seem to have less of these side effects. Individuals interested in taking niacin to help manage blood lipids should do so only after consulting with their physician. In addition, most people have to start with lower doses or consume niacin with food to lessen side effects. Nevertheless, nicotinic acid therapy appears to be an effective nutritional intervention to help manage blood lipids in addition to exercise, weight loss, and diet management.

Plant sterols and stanols (also known as phytosterols and phytostanols) are naturally occurring compounds found in plants. Phytosterols and phytostanols have been reported to inhibit cholesterol absorption in the intestine.⁴⁶ A number of studies have indicated that adding 2–3 g of plant stanol esters (expressed as free stanols) decreases total cholesterol, LDL, and triglycerides by 5–10%.^{46,50–54} Phytosterols and phytostanols have been added to margarines and other functional foods in an attempt to help increase dietary availability of these lipid lowering compounds. A recent study reported a dose-related effect of increasing dietary intake of phytosterols.⁵⁵ Therefore, another nutritional strategy that individuals with high blood lipids can employ is to consume these types of functional foods in their diet.

Omega-3 fats are considered essential fatty acids and are classified as a polyunsaturated form of fat.² There are three forms of omega-3 fats: alpha linoleic acid, eicosapentanoic acid, and docosahexaenoic acid. Research has shown that increasing dietary availability of omega-3 fatty acids can reduce LDL and triglycerides.^{46,52,56–58} The best sources of omega-3s are from fatty fish such as mackerel, lake trout, herring, sardines, albacore tuna, and salmon.² Also, the oils of tofu, other forms of soybeans, canola, walnut, and flaxseed all contain alpha linoleic acid.² For this reason, the AHA recommends consuming omega-3 containing fish two times a week. Fish oil supplements also contain omega-3s, but obtaining omega-3 fatty acids from the foods listed above is preferable to ingesting a fish oil supplement.²

Pharmacological interventions for lowering blood lipids include use of statins, fibrates, and ezetimibe.⁴⁶ Statin medications (for example, atorvastatin and rosuvastatin) have been considered as a first-line pharmaceutical approach for the treatment of dyslipidemia.^{46,59} Statins inhibit HMG-CoA reductase, resulting in a reduction in cholesterol synthesis. Clinical trials have indicated that statins can reduce LDL cholesterol by 20–60% and triglycerides by as much as 45%.⁴⁶ The most significant potential side effect for athletes is that statins have been reported to elevate creatine kinase (CK) up to ten times normal levels, resulting in myositis and in rare incidents promote rhabdomyolysis, leading to fatal renal damage. Fibrates (bezafibrate, fenofibrate, gemfibrozil, and ciprofibrate, for example) have also been reported to lower triglycerides, increase HDL levels, and have variable effects on LDL cholesterol.⁴⁶ Finally, ezetimibe is a drug that has recently been found to inhibit dietary and biliary

absorption of cholesterol in the intestine by interfering with cholesterol transport. For example, a recent study reported that ezetimibe reduced LDL by 18% while favorably influencing HDL, triglycerides, and apolipoprotein B.⁶⁰ Combination therapies of nutritional and pharmacological interventions have also been shown to be an effective means of managing dyslipidemia when exercise, weight loss, and dietary interventions fail to promote the types of clinical changes desired.

8.5 IMPLICATIONS FOR ATHLETES

There are a number of implications for athletes that the sports medicine professional needs to consider. First, one should not assume that just because athletes are engaged in intense training that they do not have dyslipidemia. Athletes who are larger, maintain higher BMIs and percent body fat, do not regularly perform endurance exercise, and/or have a significant family history should undergo screening, as a high percentage of athletes who have these characteristics have been found to have higher blood lipids. Once an athlete is found to have high cholesterol, LDL, and/or triglycerides, he or she should meet with a sports dietitian and strength and conditioning staff to discuss alterations in diet and/or training that may help lower blood lipids. For athletes with only moderately elevated LDL levels and a lower number of CHD risk factors, increased aerobic exercise, loss of body fat, and reduced consumption of high-fat and high-cholesterol-containing foods should be the initial therapeutic intervention. In addition, greater dietary intake of phytosterols and omega-3 fatty acids may help manage blood lipid levels. For those with more significant dyslipidemia, nicotinic acid supplementation and/or pharmacological approaches may need to be prescribed by the athlete's physician. Since athletes engaged in intense training may experience marked increases in CK levels, the sports medicine professional should monitor athletes taking statin drugs, particularly when they exercise in hot and humid environments. The reason for this is that one of the side effects of taking statin medications is myositis and rhabdomyolysis. Athletes engaged in intense training may therefore be at greater risk of these complications, particularly if they have sickle cell trait and/or sickle cell anemia. Finally, since many athletes are susceptible to weight gain once they retire from athletics, they may be at greater risk to observe elevations in blood lipids as they get older. Athletes should be properly educated as they complete their careers on ways to maintain an active lifestyle and consume a healthy diet in order to prevent the adverse health outcomes associated with obesity.

8.6 FUTURE RESEARCH DIRECTIONS

Additional research should evaluate the prevalence of dyslipidemia in different types of athletes; the impact of lifestyle, gender, nutrition, and pharmaceutical interventions in athletes who have dyslipidemia; and the ways to reduce the risk of athletes gaining weight and experiencing elevations in blood lipids and risk to CHD after they retire. In addition, athletes should be properly educated on ways of adjusting their physical activity and diet patterns once they retire from organized athletics so they do not experience adverse health outcomes as they get older.

8.7 CONCLUSIONS

Athletes are not immune to having dyslipidemia. Athletes who maintain higher BMI and body fat, do not participate in regular endurance exercise, or have a significant family history may be at greater risk and should therefore be screened by health-care professionals. Athletes found to have high blood lipids should increase the amount of endurance exercise they regularly perform and reduce fat and cholesterol intake in their diet. If these strategies are not effective, they should consult with their team physician and sports nutritionist to determine if additional nutritional and/or pharmacological interventions are necessary. Finally, athletes should be properly educated upon retiring from athletics on ways to minimize risk of dyslipidemia and CHD as they get older.

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9 Assessment of Protein Status of Athletes

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9.1 INTRODUCTION

9.1.1 IMPORTANCE OF PROTEIN FOR ATHLETIC PERFORMANCE

Although not considered a major source of energy, dietary protein (or more accurately amino acid) intake is important for exercise adaptation. The body functions on a simple scheme of sensing a stress and responding to minimize the disturbance caused by that stress if it is encountered again. As far as exercise and performance are concerned, the stress is designed to make the body better able to run, jump, throw, etc. The way the body—or more accurately, the individual cells—adapt to the stress is to increase the making of proteins that provide the metabolic and structural support needed to adapt to the stress. Simultaneously, proteins that might not be needed are broken down because they are energetically expensive to maintain. The turnover of proteins, which is dictated by gene expression in response to stress, determines the phenotype of the athlete. Important to this chapter is that the making of proteins requires amino acid building blocks and energy, which are consumed in the diet.¹ In a broad sense, then, we need dietary amino acids to help make the proteins that the cell requires to adapt to an exercise stimulus.

9.1.2 PROTEINS VERSUS AMINO ACIDS

Proteins are contained within and surround every cell of the body in a wide variety of forms, including enzymes, hormones, transporters, extracellular matrixes, contractile proteins, etc. In its simplest form, a protein is series of amino acids connected by peptide bonds into a polypeptide chain. Amino acids are organic compounds containing a carboxyl-carbon group attached to a nitrogen-containing group. All amino acids share the form $R-CH-NH_2-COOH$ in which the R designates the functional group, varying from a proton for glycine to double-ring structures of tryptophan. The functional R group is attached to the α -carbon in a left-handed orientation in proteins, and thus amino acids in the body are L- α -amino acids. There are over 300 amino acids; however, 23 are genetically encoded and used to build proteins in eukaryotic cells.² Twenty common amino acids are used for building new proteins in humans and animals, and eight of these cannot be synthesized and must be consumed through dietary sources. The other twelve amino acids are synthesized within the body or are only required in the diet to support the growth of children, as is the case of histidine and arginine. Amino acids that must be supplied in the diet are considered essential amino acids (EAA) compared to the nonessential amino acids (NEAA), although it should be noted that all twenty of the amino acids are essential for life. Other nomenclature attempts to clarify this fact by identifying amino acids as nutritionally indispensable or nutritionally dispensable. Additionally, two amino acids (cysteine and tyrosine) are synthesized from other EAA precursors and are considered conditionally dispensable because depletion may occur if the precursor becomes deficient. Amino acids are consumed from dietary sources as proteins and free amino acids. Long polypeptide chains are digested into short sequences or individual amino acids and are transported through the gut into general circulation.

9.1.3 PROTEIN QUALITY

The quality of a protein is its ability to meet metabolic requirements. Traditionally, protein quality was represented by the ability of a protein source to maximize growth rates, commonly in rats but also in humans. This view, however, focused on amino acids being used for protein synthesis and neglected other roles of amino acids such as nutrient signaling or neurotransmitter production. The quality of a protein is characterized by the amino acid content and availability in the protein source. Classifying a protein based on its quality is useful for nutritional recommendations; however, empirically determining quality is difficult and has been revised over many years. A simple method for calculating protein quality is to compare the amino acid pattern against a reference protein source that is known to promote growth, such as egg or milk proteins. Although simple, the method does not consider the ability of the amino acids to be absorbed and used in the body (bioavailability) or the amino acid requirements of the organism. In order to address these issues, protein quality can be determined by the ability of a protein source to promote growth rates, commonly using rats.³ The amount of protein fed to the rat is divided by the weight gained to calculate a protein efficiency ratio (PER). A greater amount of weight gained for a given weight of food will produce a higher PER. Although the PER provides an indication of protein quality, the differences in growth rates between strains and different metabolic characteristics from humans limit the reliability and applicability of the data for human health recommendations.

In 1991, a joint committee of the World Health Organization and the Food and Agriculture Organization (WHO/FAO) recommended an amino acid scoring calculation to evaluate protein quality.⁴ The score represents the ability of a protein source to provide individual amino acids compared to the amino acid requirements of a human reference population, and corrects that score for the digestibility of the protein source. It assumes that amino acids must be provided in sufficient quantities to meet metabolic demands, and that a limitation of any one amino acid would impair processes, such as protein synthesis. Since EAA cannot be synthesized endogenously, the individual EAA that is consumed in insufficient quantity to meet metabolic demands is considered the limiting amino acid. The required intake of the limiting amino acids is based on amino acid balance studies using preschool children as a reference population, and the amino acid score of a protein is calculated as

$$\text{amino acid score} = \frac{\text{mg of amino acid in 1 gm test protein}}{\text{mg of amino acid in reference protein}}$$

The digestibility of the protein is calculated from the ingested nitrogen content compared to the fecal nitrogen content, corrected for required nitrogen loss determine on a protein-free diet. Thus, the digestibility of a protein is

$$\text{digestibility}(\%) = \frac{I - (F - Fr)}{I} \times 100$$

where I is the intake of nitrogen, F is fecal nitrogen content, and F_r is required fecal nitrogen content determined on protein-free diet. The digestibility is used to adjust the amino acid score to calculate the protein digestibility-corrected amino acid score (PDCAAS):

$$\text{PDCAAS}(\%) = \text{digestibility} \times \text{amino acid score}$$

The PDCAAS attempts to classify protein sources based on their amino acid content and bioavailability.

A low-quality protein limits protein utilization and must be consumed in higher quantity or in combination with high-quality proteins to meet protein requirements. In contrast, a high-quality protein can more readily meet protein demands and can be consumed in lower quantities. Additionally, protein quality is decreased if amino acids have limited bioavailability due to limited digestibility, absorption, or incorporation into new proteins. Factors that contribute to limited bioavailability can be intrinsic to the protein source, develop with disease or aging, or be introduced during the manufacturing processes. For example, heating a protein source in the presence of reduced sugars can cause a Maillard reaction that creates cross-linkages between amino acid residues and decreases protein quality.⁵

The PDCAAS classifies protein sources based on their protein quality; however, the WHO/FAO and others suggest that the PDCAAS is based on assumptions that limit interpretation and should be revised.^{4,6,7} Criticism of the PDCAAS score suggests that using preschool children as a reference population may not be representative of all populations and their amino acid needs.⁶ In particular, older people appear to be less sensitive to circulating amino acids,⁸ thus their dietary requirements are elevated due to changes in sensitivity and not necessarily metabolic demand.⁹ Additionally, the protein digestibility is calculated from fecal values; however, fecal values are the results of net contributions of the entire digestive tract. It was suggested that the amino acid absorption that is relevant for protein requirements occurs within the ileum, and additional amino acid consumption by bacteria within the colon will overestimate the true nitrogen digestibility.⁶ Another limitation of the PDCAAS is that protein quality can never be greater than 100% compared to the reference population because amino acid scores greater than 1.0 are truncated and digestibility cannot exceed 100%.⁷ Thus, protein sources are compared against the limiting amino acid regardless of the other EAA. Two protein sources with the same limiting amino acid content and digestibility will have the same PDCAAS but could vary in EAA content. The PDCAAS is limited because it does not consider that different protein sources could be complementary to each other by providing EAA that are deficient in one of the sources.⁶ Energy balance is also not considered when determining overall protein quality but appears to be very important when evaluating protein metabolism.^{7,10} Age, gender, physiological status, and disease states are also not considered and can impact on the ability of protein intake to meet protein demands. In summary, the PDCAAS provides a standardized measure of protein quality; however, its limitations must be recognized and addressed.

A quantitative measure of protein quality is beneficial for classifying protein sources and to ensure adequate protein intake for people on strict diets. For athletes and those on a restricted diet, consuming a high-quality protein may be beneficial

because it limits excess caloric intake that comes with added protein. Different protein sources can also influence training adaptations even though the same quantity of protein is consumed.^{11,12} Therefore, a high-quality protein, such as those from egg or milk sources, may be beneficial to cutting weight while maintaining lean body mass.

9.1.4 ADAPTATION TO EXERCISE: SIGNALS, TRANSCRIPTION, AND TRANSLATION

Exercise is a strong signal to induce changes in protein content, including those more pronounced like muscle hypertrophy or those subtler like altered mitochondrial proteins. Regardless of their different types or functions, the production of proteins from individual amino acids is a highly coordinated and multifaceted process. As described above, the general process is sensing stress signals to induce adaptations to minimize future perturbations. A single exercise stimulus contains a multitude of diverse signals, including energetic stress, nutrient deficiency, mechanical loading, and circulating hormones. The nucleus integrates multiple signals and responds by encoding mRNA transcripts from the appropriate DNA sequences, a process termed transcription. The mRNA transcript contains the coding template for the amino acid sequence of the protein. Translation is the process by which RNA polymerase enzyme complexes read the mRNA transcript and attach amino acids together in the proper order. Following translation, the new polypeptide strand must be properly folded into its final structure to allow proper function. The endoplasmic reticulum is a major site for protein folding of the cell and contains the necessary environment and machinery to process nascent polypeptide chains.¹³

It is necessary to recognize that not every signal will lead to the expression of a fully functional protein. Multiple independent regulation steps within signaling, transcription, and translation pathways coordinate protein synthesis. A signal can also have diverse responses to coordinate a single goal. The regulation steps may be specific, such as turning on a specific transcription factor for a family of gene, or more general, such as limiting the overall protein synthesis machinery of the cell.

Short-term signals are often mediated by changing the phosphorylation state of a protein that subsequently alters the activity or function of a protein. For example, eukaryotic elongation factor-2 (eEF2) is a regulatory protein that decreases protein synthesis when phosphorylated. Increased phosphorylation of eEF2 was shown following a single minute of exercise and remained elevated through 90 minutes of endurance exercise.¹⁴ Specific mRNA transcripts also increase following exercise, but the time course appears to be longer than phosphorylation events with some transcripts increased immediately postexercise and peaking between 2 and 8 hours postexercise.^{15–17}

The time course and signaling characteristics between initial stimulus (such as exercise) and eventual synthesis of proteins is important when attempting to maximize adaptations to exercise. Initial increases in translational activity appear to mediate acute adaptations to exercise and are followed by increased transcriptional activity to create more mRNA.¹⁸ The increase in mRNA provides additional transcripts that can be subsequently translated into new proteins. The combination of rapid translational events and delayed transcriptional events causes the resultant increase in protein content. Recommendations to maximize recovery from exercise

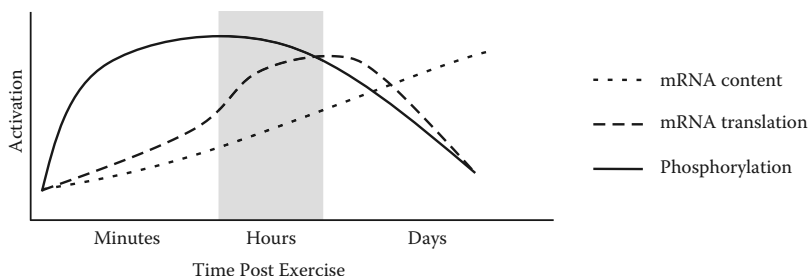


FIGURE 9.1 Exercise activates protein synthesis through rapid changes in phosphorylation states of regulatory proteins that allow increased translation of existing mRNA transcripts. Increased transcription activity will increase mRNA content over longer time periods. The shaded region represents the optimal timing for maximal stimulation of protein synthesis during initial recovery period.

are based on these time-course characteristics. For example, when the duration for elevated mRNA transcript is compared against the increased activity of protein synthesis machinery, then we can identify a time period when maximal adaptations most likely occurs (Figure 9.1). This concept is very important when discussing the timing of nutrient intake around exercise to maximize recovery.

9.2 PROTEIN METABOLISM

9.2.1 PROTEIN TURNOVER

The total protein content in a cell, tissue, or organism is the net contribution of the synthesis of new proteins and the breakdown of existing proteins. The combination of these two processes (protein turnover) is necessary for maintaining cell processes by replacing older proteins with newer proteins. The renewal of proteins promotes cell function by allowing older proteins that may have diminished function to be replaced by new proteins that have improved function. The overall content of proteins may not change, but the quality and function of the proteins are improved. The rate at which a protein is synthesized and degraded is referred to as its half-life ($t_{1/2}$) and often determined by its physiological task. Proteins that are involved in regulatory roles, such as transcription factors or hormones, can have higher turnover rates ($t_{1/2} < 30$ min) than contractile proteins such as myosin heavy chain ($t_{1/2} \sim 54$ days).¹⁹ Either accumulation or loss of the protein occurs when the contribution of synthesis and breakdown are not in equilibrium. These two processes will be discussed separately.

9.2.1.1 Protein Synthesis

Protein synthesis is the translation of mRNA transcripts to combine individual amino acids (specifically amino-acyl tRNA) into polypeptide chains. These nascent peptides are then folded into their proper structure by chaperone proteins and are transported to their appropriate destination such as the plasma membrane for transmembrane receptors, released into the cytosol for intracellular signaling proteins, or secreted as in the case of peptide hormones. Interestingly, the machinery required

for protein translation is made of proteins (for example, ribosomes and chaperones); thus cells must maintain basal rates of protein synthesis to maintain the proteins necessary for translation.

9.2.1.2 Protein Breakdown

Protein breakdown is the removal of existing proteins and yields either short chains of amino acids or individual amino acids. These can then be used by the cell as needed, including for translation of new proteins, oxidation for adenosine-5'-phosphate (ATP) production, or export and transport to other cells. Protein breakdown occurs through a host of enzymatic processes. Peptide chains are cleaved by proteases into short peptide chains, which are then digested into individual amino acids by endoproteases. These proteases can be free within the cell or contained within intracellular organelles that serve as major sites for protein degradation, such as the lysosome and proteasome. The proteasome pathway uses short amino acid sequences (called ubiquitin) and a series of enzymatic reactions to target proteins for degradation within the proteasome. Polyubiquitination of proteins can occur and accelerate proteolysis.

Although nutritional research and recommendations often focus on protein synthesis pathways, protein breakdown is a necessary and important process for maintaining cell function and the health of the organism. As indicated above, protein breakdown contributes to protein turnover and promotes cellular function by replacing older proteins with newly synthesized proteins. Additionally, protein breakdown can provide amino acids to the pool of intracellular amino acids. This is especially important when considering essential amino acids (EAA), which cannot be synthesized and enter the intracellular amino acid pool through either dietary sources or release from protein breakdown. If dietary EAA are insufficient to meet the needs of protein synthesis, then existing proteins can be degraded and supply the necessary amino acids. This concept indicates that skeletal muscle can serve as a reservoir to store and release amino acids depending on their availability and metabolic demands.²⁰

9.2.1.3 Net Balance

The net balance of proteins is the total contribution of protein synthesis and breakdown and is commonly expressed as

$$\text{Net Balance} = \text{Protein Synthesis} - \text{Protein Breakdown}$$

If the synthesis and breakdown of proteins are occurring at equal rates, then the net balance will be zero and no gain or loss of protein occurs ([Figure 9.2](#)). It is possible for both synthesis and breakdown to be elevated and cause an increased protein turnover without net gain or loss. If protein synthesis exceeds protein breakdown, then net protein balance will be positive and indicate an accumulation of proteins. Conversely, if protein breakdown exceeds protein synthesis, then the net balance will be negative and indicate a loss of proteins. When considering net balance, it is necessary to understand the context in which the parameter is being evaluated. For example, if whole-body protein synthesis and breakdown are being evaluated, then net balance reflects the entire change in the protein pool of the body. It is possible to evaluate specific limbs and tissues in order to determine local changes to net

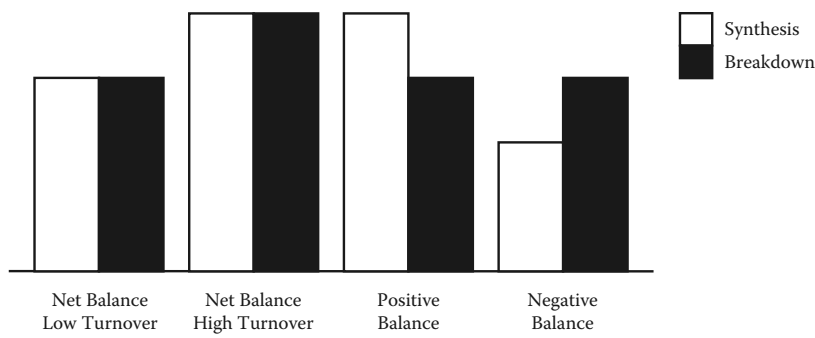


FIGURE 9.2 Schematic contribution of protein synthesis and breakdown to net balance and turnover.

balance that may not be detected with whole body measures. For example, whole-body protein turnover did not change following a resistance exercise session in young men.²¹ However, a limb-specific model showed increased protein synthesis in the legs of young men, with a larger increase of protein breakdown that resulted in a net negative protein balance.²² These studies support that protein metabolism can be evaluated generally (whole-body protein balance) or locally (protein balance within the leg), and that different conclusions may be drawn based on the study design. Methodological advances have allowed protein synthesis rates to be determined for individual muscle proteins.²³ Future advances may allow protein breakdown rates to be determined and allow determination of net balance of individual proteins. Thus, net balance provides insight into the overall contribution of protein synthesis and breakdown and allows a more complete evaluation of protein metabolism than either individual process.

Net balance can be somewhat limiting in the absence of information about synthesis and breakdown. As discussed above, synthesis and breakdown can vary without changing net balance. Further, net balance does not consider that a high rate of protein turnover allows rapid amplification of protein content following changes in the rate of protein synthesis or breakdown, or a combination of both. One such example is found with the transcription factor hypoxia inducible factor-1 α (HIF-1 α), which degrades rapidly after it is synthesized and maintains a constant protein content. However, hypoxia produces a signal to stabilize HIF-1 α and causes a rapid accumulation of protein to promote the transcriptions of genes and subsequent adaptation.²⁴ Thus, the net balance of a protein does not reveal information about synthesis or degradation kinetics or their regulation.

Protein synthesis appears to respond to stimuli and influence net balance to a greater extent than breakdown. For example, Paddon-Jones et al. used leg-specific measures and showed that 28 days of bed rest caused muscle atrophy with decreased protein synthesis but no change to protein breakdown.²⁵ Furthermore, older adults have decreased rates of muscle protein synthesis but no difference in protein breakdown as compared to younger people during resting conditions.²⁶ These findings do not diminish the importance of degradation pathways but suggest that changes to net protein balance are driven primarily by changes to rates of protein synthesis.

9.2.2 FATES OF AMINO ACIDS OTHER THAN PROTEIN SYNTHESIS

Amino acids are commonly considered in reference to protein synthesis; however, they can serve as precursors for others processes, including gluconeogenesis, synthesis of neurotransmitters, intermediates in the Krebs's cycle, and ATP production (Figure 9.3). Before individual amino acids can be used for such pathways, the amino group is removed by transamination. Transamination is reversible enzymatic reaction catalyzed by specific aminotransferases that transfer the amino group from an L- α -amino acid to an α -keto acid. Removing the amino group converts an α -amino acid to its α -keto acid form. Transamination does not degrade an amino acid but converts an L- α -amino acid to an α -keto acid and uses the nitrogen group to convert another α -keto acid to an L- α -amino acid. The process can be reversed to produce the twelve nonessential amino acids from their α -keto acid, which are found as intermediates within glycolysis and the Krebs's cycle.

The α -keto acids can be incorporated into the Krebs's cycle to expand the pool of intermediates (a term called *catapleurosis*) and can be used as substrates for eventual production of ATP by oxidative phosphorylation. For example, L-glutamate can be transaminated to form α -ketoglutarate and used to produce NADH in the Krebs's cycle by the α -ketogutarate dehydrogenase complex. The α -keto acid products can also be used as gluconeogenic precursors, such as L-alanine being transaminated to pyruvate for eventual formation of glucose.

Amino acids can also be catabolized by amino acid oxidase enzymes to produce NADH, a process termed oxidative deamination. Glutamate is a common amino group acceptor from transamination and is therefore a primary amino acid

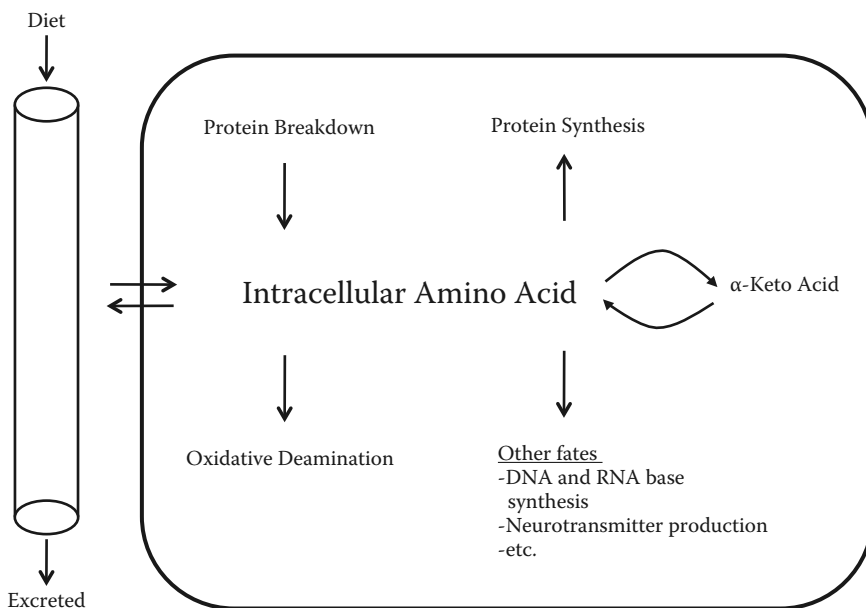


FIGURE 9.3 Intracellular fates of amino acids.

used for oxidative deamination in mammalian tissue. The liver contains many amino acid oxidases and is a major site for oxidative deamination. However, other tissues can oxidize amino acids, which must be considered when evaluating the fate of amino acids. For example, skeletal muscle can use leucine but not phenylalanine as an energy source. Thus, the fate of leucine uptake into skeletal muscle cells includes protein synthesis and oxidation, whereas phenylalanine will be used only for protein synthesis. The metabolism characteristics of amino acids must be considered when evaluating protein turnover methodology.

9.2.3 SKELETAL MUSCLE PROTEIN TURNOVER

Skeletal muscle protein turnover is especially important when considering athletic performance because of its size, its important role in locomotion, and its role in energy balance. Small changes in turnover relative to a given mass of tissue will be amplified by the sheer size of tissue to large absolute rates. This concept is important in regards to energy balance because skeletal muscle is a highly metabolic tissue in terms of absolute rates. Other tissues, such as liver, have higher relative rates of energy consumption and protein turnover, but the large size of skeletal muscle is a greater absolute contributor to whole-body protein turnover and metabolic rate.²⁷ Since amino acids do not have a specific storage site such as adipose tissue for fatty acids, synthesizing amino acids into skeletal muscle proteins may be the only way to “store” amino acids during times of excess (such as feeding) to be used in times of need (such as fasting). Therefore, investigations of protein turnover and nutritional requirements often focus on skeletal muscle because of its size and dynamic nature, and its importance in the determination of exercise performance or health.

9.2.4 TURNOVER OF PROTEIN IN OTHER TISSUES

All tissues synthesize and degrade proteins continually, and some tissues have very high rates of protein turnover. Loss of skin, hair, blood, sweat, and cells lining the intestinal tract are continual sources of protein loss from the body that need to be replenished. Depending on the involved processes, the rates of protein turnover can be very high relative to the tissue size. The liver is a major source of secreted proteins such as hormones and lipoproteins and has high rates of protein turnover. In fact, the liver accounts for ~20% of whole-body oxygen consumption although it is only ~2% of the body's mass.²⁷ Contrasted against skeletal muscle that is ~42% of the body's mass and consumes ~20% of total oxygen consumed, it is apparent that smaller tissues can be highly metabolic.²⁷ Skeletal muscle receives much focus when considering protein requirements and turnover, but that focus should not detract from the importance and contribution of other tissues to protein metabolism.

9.3 IMPORTANCE OF ENERGY BALANCE

Protein synthesis is the greatest energy-consuming process of the cell, and under resting conditions protein turnover contributes to about ~20% energy requirements.^{27,28} Protein synthesis requires the hydrolysis of four high-energy phosphates per peptide

bond: two ATP for charging the aminoacyl tRNA molecule and two guanosine-5'-triphosphates (GTPs) for entry and translocation on active sites of the ribosome complex. Additionally, the formation of the ribosome complex requires GTP hydrolysis to form peptide bonds between amino acids of the lengthening protein. In addition to peptide bond formation, protein synthesis requires RNA synthesis and transport of amino acids across membranes into the cell, all of which are ATP-consuming processes. Thus, the energetic calculation of protein synthesis needs to evaluate these processes as well. A single protein can be made of hundreds of peptide bonds; thus protein synthesis requires a continual supply of energy. As an example, hemoglobin contains 574 amino acids and requires ~2300 high-energy phosphates to form all of its peptide bonds. Since red blood cells contain many hemoglobin molecules and are being continually replaced, one can imagine that much energy is required to maintain hemoglobin protein levels, let alone the other proteins found within a red blood cell. Even synthesis of a smaller protein such as pro-insulin, the precursor to secreted insulin, with 119 peptide bonds has large energy requirements (~476 ATP).

Protein breakdown is also an ATP-consuming process, although to a lesser extent than protein synthesis. The ubiquitin–proteasome pathway consumes approximately four ATP per degraded protein.²⁹ The combined energetic costs of protein breakdown and synthesis using protein turnover and metabolic rate have been estimated at 1.04 kcal for each gram of protein,²⁸ with 1–2% of all proteins within the body replaced daily. Conditions with elevated protein turnover will increase protein intake requirements along with energy costs. For example, burn victims have a hypermetabolic state characterized by increased resting energy expenditure and higher rates of protein synthesis. About 25% of the increase in energy expenditure is due to the energy requirements of increased protein synthesis.³⁰

Energy balance is the difference between energy consumed and energy expended and has a critical contribution to protein metabolism. The synthesis and breakdown of proteins are ATP-consuming processes, and it has been recognized since the first part of the twentieth century that protein balance is strongly influenced by energy balance.³¹ In 1954 Calloway and Spector performed an important review of available nitrogen balance data and concluded that at a fixed adequate protein intake, energy level is the deciding factor in nitrogen balance.³² Their paper also established minimal protein or energy intakes at which increasing one or the other has no influence on nitrogen balance. Two additional studies from Calloway's laboratory demonstrated that nitrogen balance is better maintained when a caloric deficit is induced from physical activity rather than diet,¹⁰ and that exercise training actually increases the ability to maintain nitrogen balance at a given energy balance and protein intake.³³ Finally, Chiang and Huang showed that increasing levels of positive energy balance (+15% and +30%) caused greater nitrogen retention when provided a given amount of protein.³⁴ It is now evident that energy-sensing pathways can regulate protein turnover, in particular through regulating protein synthesis. For example, one negative regulator of protein synthesis is AMP-activated protein kinase (AMPK). AMPK responds to low-energy states by activating energy producing pathways and impairing energy-consuming pathways including protein synthesis.³⁵ Additionally, the mammalian target of rapamycin (mTOR) complex is a primary regulator of protein synthesis and directly responds to

decreased cellular energy status by decreasing protein synthesis.³⁶ In summary, energy balance is an important regulating factor for protein turnover.

Energy and protein levels supplied by a Western diet are typically in excess of requirements; therefore energy intake is commonly sufficient to supply protein turnover needs. There are examples of negative energy balance that could be detrimental for protein balance such as with aging, dietary restrictions for weight loss, total parenteral nutrition, or exercise without increased energy intake. These conditions may cause an undesired loss of muscle mass through decreased energy availability. The recognition that protein turnover is energetically costly is not novel but is commonly overlooked when evaluating protein requirements.¹

9.4 DETERMINATIONS OF PROTEIN STATUS

There are various methods to assess protein status of an individual. Some methods evaluate the organism as a whole (for example, nitrogen balance) and others evaluate tissues or even individual proteins (such as fractional synthesis rates using stable isotope tracers). Each method has benefits, limitations, and assumptions that should be considered when interpreting the results. A brief discussion of common methods for evaluating protein metabolism is included; however, additional resources provide more in-depth discussions of the derivations, applications, and assumptions of each method.³⁷

9.4.1 LABORATORY DETERMINATIONS OF PROTEIN STATUS

9.4.1.1 Whole Body Protein Turnover

9.4.1.1.1 Nitrogen Balance

Amino acids are the primary carrier of nitrogen in the body, so when we are considering amino acid and protein metabolism, we are ultimately considering nitrogen metabolism. Nitrogen balance compares the amount of nitrogen consumed versus the amount lost and gives an indication if protein is being retained or excreted on a net basis. If the amounts of nitrogen consumed (N_{in}) and excreted (N_{out}) are known, then nitrogen balance ($N_{balance}$) is expressed as

$$N_{balance} = N_{in} - N_{out}$$

Nitrogen content is converted to protein content by the relationship that 6.25 grams of protein contain 1 gram of nitrogen (multiply grams of N by 6.25 to determine grams of protein). N_{in} is controlled by dietary intake since nitrogen is consumed only as proteins or amino acids. Diet records can be analyzed to estimate nitrogen consumption; however, the records or analysis programs may not be fully accurate. Precise determination of nitrogen intake often requires prepared diets with known amounts of nitrogen content. Additionally, oral or intravenous delivery of amino acid solutions improves control of N_{in} .

N_{out} is much more difficult to quantify than N_{in} . Fundamentally, N_{out} represents the loss of nitrogen from the body. However, nitrogen is lost from the body in many forms, including urine, feces, hair, blood, sweat, and cells from the skin

and gastrointestinal tract. Thus a precise determination of N_{out} requires that each of these sources of nitrogen loss be collected and quantified. Fortunately, previous studies have taken labor-intensive measures (such as loss during tooth brushing) and attempted to fully quantify nitrogen loss.³⁸ The results indicate that nitrogen loss from sources other than urine and feces are minimal (~500 mg/day) and can be accounted for with a mathematical correction factor. Urine and feces are the major routes of nitrogen loss and can be analyzed for nitrogen content, although urine is the most commonly measured source of excreted nitrogen. Using urinary nitrogen content and standard correction factors to account for nonurine losses, daily nitrogen balance can be expressed as

$$N_{\text{balance}} = N_{\text{in}} - (N_{\text{urine}} + 5 \text{ mg/kg} + 2000 \text{ mg})$$

with miscellaneous nitrogen losses accounted by 5 mg per kg body weight and fecal loss at 2000 mg per day.

The nitrogen balance technique allows insight into the overall net contribution of protein synthesis and breakdown in the body. Nitrogen balance is a relatively simple and noninvasive method for evaluating whole-body protein status over longer periods such as days to weeks³⁹ and is useful as an overall evaluation of protein gain or loss. Although less precise than other methods, nitrogen balance can be used with other measurements to provide a more complete evaluation of protein balance.^{40,41}

9.4.1.1.2 *Isotopes in Metabolic Research*

The use of stable isotopes in metabolic research has allowed a greater insight into the kinetics behind observable changes. It is apparent that the absolute quantity of a substance (such as muscle protein content) is the sum total of synthesis and degradation pathways and that the movement of substrates through these pathways, termed flux, yields more information than just changes in total quantity. Isotopes are used to determine the kinetics (rate of movement) of metabolic pathways. Isotopes are atomic elements that contain the same number of protons but vary in the number of neutrons and subsequently their atomic mass. These isotopes can be stable or experience radioactive decay. For example, carbon exists primarily with twelve neutrons and is referred to as ^{12}C but can also exist as stable (^{13}C) and radioactive (^{14}C) isotopes. Radioactive isotopes were traditionally used in metabolic research due to their analytical sensitivity; however, technological advances in mass spectrometry (the primary analytical tool for stable isotopes) have brought stable isotopes to the forefront of isotope research. Amino acids contain carbon, nitrogen, and hydrogen atoms that can be isotopically labeled. Isotopic tracers nomenclature identifies the atom that is labeled and its position within the molecule. For example, a leucine molecule that is labeled with a single ^{13}C as the first carbon is 1- ^{13}C -leucine. Multiple isotopes can be identified such as [6,6]- $^2\text{H}_2$ -glucose, which has two deuterium atoms on the sixth carbon of glucose, or ring- $^{13}\text{C}_6$ -phenylalanine containing ^{13}C for all six carbons in the ring structure of phenylalanine. Molecules that contain isotopes are considered to be enriched tracers and are treated the same as the nonenriched molecules they resemble. Tissue samples (for example, blood or muscle biopsy samples)

are analyzed for tracer content by determining the ratio of tracer to tracee, called the enrichment of the sample.

The concept of isotope tracer research can be represented by dilution of a dye into a bathtub with a constant level of water. The dye represents the infusion of a tracer and the tub of water represents the tracee content of the entire body. Water flows into the tub via the faucet and drains out at an unknown rate. If a known amount of dye is dropped into the tub, the dye will be diluted due to the rate of water flowing through the tub. Using the rate of infusion of dye into the tub, we can then calculate the flow rate of the water through the tub. This concept of tracer dilution is central for isotope methodology.

Amino acid tracers that are either orally consumed or intravenously infused have the same fates as other amino acids, namely, to be incorporated into proteins, oxidized as energy substrates, or excreted from the body. If the body is considered a single pool of amino acids, then amino acids enter from either exogenous (dietary and infusion) or endogenous sources (protein degradation and amino acid synthesis). Since essential amino acids (EAA) are not synthesized endogenously, then the entry of an EAA into the body pool is restricted to consumption or infusion and release from protein breakdown (Figure 9.4). Since tracers exist in very small quantities within the body, the amino acids released from proteolysis will be mostly unlabeled and will dilute the amount of labeled tracer that was infused. Thus in the fasted state, the dilution of the labeled tracer is used to determine the rate of appearance of amino acids from protein breakdown.

Amino acids that leave the pool can either be oxidized as an energy substrate or be used by nonoxidative pathways, which is primarily protein synthesis. If an amino acid tracer with a carbon isotope is consumed or infused, then the carbon will be removed during oxidation and exhaled in the breath as labeled carbon dioxide. Breath samples are collected and analyzed for labeled carbon dioxide to determine the amount of amino acid oxidation. The total rate of disappearance is known from

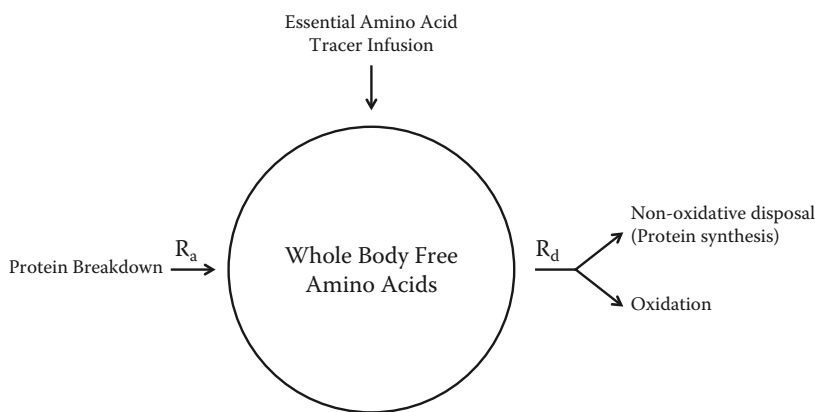


FIGURE 9.4 Single pool model. Under fasted conditions with an essential amino acid tracer, the source of amino acids for tracer dilution is protein breakdown. The flow of amino acids entering the pool (R_a) equals the flow leaving (R_d).

blood samples and the rate of oxidation is known from breath samples. The primary nonoxidative fate of amino acids in skeletal muscle is protein synthesis and is represented as nonoxidative disposal. The value is calculated from the difference between the total rate of disappearance and oxidation.

In practice, 1-¹³C-leucine is intravenously infused in fasted individuals with venous blood and breath samples obtained periodically (usually hourly). 1-¹³C-leucine is commonly used as the tracer of choice for whole-body protein turnover characteristics because it yields ¹³CO₂ when oxidized and its major nonoxidative fate is protein synthesis. It is also an EAA, so in the fasted state the appearance of leucine can only come from protein breakdown. The dilution of the infused tracer by unlabeled leucine represents protein breakdown and is calculated as

$$R_a = \frac{F}{E_{\text{KIC}}}$$

where F is the infusion rate of the tracer and E_{KIC} is the isotopic enrichment of α -ketoisocaproate (KIC). KIC is the α -keto acid produced from leucine transamination. Since transamination occurs intracellularly and then KIC is released into circulation, circulating KIC represents the intracellular content of leucine. The R_{ox} of leucine is calculated by measuring the ¹³CO₂ content in breath samples and KIC enrichment in blood:

$$R_{\text{ox}} = \frac{{}^{13}\text{CO}_2}{E_{\text{KIC}}}$$

Since the whole-body protein model assumes a steady state in which the rates of amino acid disappearance (R_d) and appearance (R_a) are in equilibrium

$$R_d = R_a$$

$$\text{NOLD} + R_{\text{ox}} = R_a$$

$$\text{NOLD} = R_a - R_{\text{ox}}$$

where nonoxidative leucine disposal (NOLD) represents whole-body protein synthesis.

9.4.1.2 Skeletal Muscle Protein Turnover

9.4.1.2.1 3-Methyl-Histidine

Modifications to amino acids occurring after protein translation are used to evaluate characteristics of protein turnover in skeletal muscle. Common posttranslational modifications include hydroxylation and methylation of amino acid residues within proteins. Because tRNAs do not exist for these modified amino acids,⁴² protein breakdown releases the modified amino acids and they are not recycled into other proteins. Following protein breakdown, the modified amino acids are released into circulation and are eventually excreted. Thus, the presence of these

posttranslational modified amino acids in blood or urine is representative of protein breakdown.

One such modification is methylation of histidine to form 3-methyl-histidine. Skeletal muscle actin and myosin proteins contain much of the 3-methyl-histidine (3-MH) content in the body, and therefore the appearance of 3-methyl-histidine in urine can be used to evaluate skeletal muscle protein breakdown, specifically myofibrillar protein breakdown.⁴³ However, the content in urine is from proteolysis of the entire body, influenced mostly by skeletal muscle (~75%) but also other sources such as rapidly turning over gut and skin proteins.⁴⁴ Localized sampling techniques such as arterial-venous (A-V) differences and microdialysis can limit the contribution of 3-MH from nonskeletal muscle and evaluate proteolysis over more specific tissues.^{45,46} A-V differences between the 3-methyl-histidine concentration in the blood supplying the muscle (arterial) and blood draining the muscle (venous) determine the protein breakdown of the tissue bed. Increased proteolysis will release more 3-MH into venous blood and increase the A-V difference. A-V differences provide more localized values but are still influenced by all the tissues that release 3-MH into the sampled vein. Microdialysis procedures allow a further degree of localization. The technique involves inserting a small tube (called a probe) through the tissue of interest. A section of the probe within the tissue contains a semipermeable membrane that allows molecules to pass into the lumen of the probe and be collected. If the probe is inserted into skeletal muscle, then that specific tissue is the primary source of 3-methyl-histidine. In summary, posttranslational modifications to amino acids can provide insight into protein breakdown, but the source of the compound and the site of sampling need to be considered.

9.4.1.2.2 Fractional Synthesis Rate

A widely used method uses stable isotopic tracers to determine the fractional synthesis rate (FSR) of proteins. The FSR indicates the fraction of the protein pool that is synthesized over a given time, usually represented at %/hour. By definition, proteins with a higher FSR are being synthesized at a faster rate. It is important to note that FSR represents a fraction of the protein pool being synthesized. In order to obtain absolute synthesis rates, the FSR is multiplied by the pool size of the protein. Thus, a small protein pool with a high FSR rate may have a lower absolute synthesis rate than a large protein pool with a lower FSR.

Measuring FSR is based off a precursor-product relationship in which amino acids are the precursor building blocks to form protein products. Amino acid isotopic tracers are infused into circulation and are incorporated into the intracellular amino acid pool that is used to synthesize new proteins. Tissue biopsy samples are then analyzed for tracer content and FSR is calculated as

$$\text{FSR} = \frac{E_{t2} - E_{t1}}{E_p(t_2 - t_1)}$$

where E_{t2} and E_{t1} are the isotopic enrichments of the protein product at two time points, E_p is the enrichment of the precursor, and t_2 and t_1 are the times for tissue

sampling. FSR is the change in product enrichment divided by the average precursor enrichment during the sample time period. Specificity of FSR is limited by the ability to sample a tissue or isolate a specific protein. For example, a muscle biopsy sample that does not undergo further separation techniques will yield mixed muscle FSR.⁴⁷ Additional steps can separate various protein subfractions such as myofibrillar, sarcoplasmic, and mitochondrial proteins to determine the FSR of each group of proteins.⁴⁸ Even further analytical steps can determine the FSR of individual proteins and have identified that different proteins within subfractions have varied synthesis rates.²³ The concept of subfractions is further discussed in [Section 9.4.1.2.5](#).

Continuous infusions of stable isotopes are commonly used over a period of hours (4–10) to allow sufficient tracer incorporation into the protein product during an isotopic steady state such that E_p is constant. However, a single large flooding dose of isotope can be used to shorten the time period necessary (30 to 90 minutes) to allow sufficient label incorporation. Both methods rely on the precursor–product relationship in which the true precursor for protein synthesis must be known. Although amino acids are commonly considered the building blocks for proteins, the final form that is incorporated into polypeptide strands is charged aminoacyl-tRNA. Thus, the true precursor for protein synthesis is tRNA, which is very difficult to measure in small muscle samples such as human biopsies.⁴⁹ The flooding dose technique avoids this problem because all amino acid pools are flooded with tracer such that sampling any of the pools (such as blood) represents the isotopic enrichment of the true precursor. The constant infusion technique requires that surrogate measures such as intracellular or plasma enrichment be used for the precursor enrichment. Transamination products released into circulation provide a useful representation of intracellular amino acid content. A common example is using plasma KIC to represent intracellular leucine.⁵⁰ Leucine is transaminated within cells to form KIC, which is subsequently released into blood. The relationship is reciprocal, meaning that labeled KIC can be infused, transaminated to leucine, and used for protein synthesis. In this situation, labeled leucine is also released into circulation and is the precursor for FSR calculations.

The flooding dose technique requires much less time and provides a better representation of the true precursor, and it was widely used to calculate FSR. However, it was realized that providing flooding doses of amino acids, in particular essential amino acids, stimulated the making of proteins through a feeding-like effect.^{51,52} These findings were important because they identified that (1) the flooding dose technique can artificially elevate FSR, and (2) essential amino acids can stimulate protein synthesis. Therefore, the constant infusion technique is more commonly used to determine FSR, although it must be recognized that long study designs (4–10 hours) may not fully represent a physiological steady state or acute effects following an intervention.

Fractional breakdown rate (FBR) is determined using a reversal of FSR, meaning that the final product is free amino acids and the precursor is bound proteins.⁵³ Tracers are infused initially to reach isotopic steady state in circulation then the infusion is stopped. The tracer is diluted by the release of unlabeled amino acids from protein breakdown. Regular blood samples are used along with one or two muscle biopsies to plot the rate of plasma and intracellular tracer dilution. An essential amino

acid tracer must be used since the method assumes that unlabeled amino acids are coming from protein breakdown and not endogenously synthesized. Additionally, the protein source of amino acids cannot be determined, and thus the FBR of sub-fractions or individual proteins cannot be determined using this method.

9.4.1.2.3 Two- and Three-Pool Models

The concept of body pools is an attempt to model the movement of amino acids throughout the body. The simplest model is a single pool that is used for whole-body protein turnover and provides information about overall protein turnover; however, regional characteristics of amino acid metabolism are not known (see [Section 9.4.1.1](#)). Two- and three-pool models allow greater understanding of amino acid transport characteristics, although it is apparent that modeling the body as two or three pools of amino acids is still simplistic.⁵⁴ The concept of two- and three-pool models is based on the Fick principle of tissue substrate use in which

$$\text{Tissue uptake} = \text{Blood flow} \times (\text{artery} - \text{venous difference})$$

$$\text{Tissue outflow} = \text{Blood flow} \times (\text{venous} - \text{artery difference})$$

Adolph Fick initially used the relationship between A-V differences in O₂ concentration to calculate blood flow across the lungs⁵⁵; however, the relationship between blood flow and tissue utilization can be readily applied to other circulating substrates. The two-pool model compartmentalizes amino acids into (1) the arteries that supply tissues and (2) the veins that drain tissues (Figure 9.5). The concept is similar to nitrogen balance because the difference between inflow (artery) and outflow (vein) provides a representation of net balance across a tissue. A greater net uptake of amino acid is indicative of protein synthesis, whereas a net release indicates protein breakdown.

In the case of two-pool models, the tissue of interest is identified along with its appropriate artery and vein. Commonly the skeletal muscle of the leg is of primary interest; therefore the femoral artery and vein are catheterized for periodic blood samples. Blood flow and amino acid concentrations determine the total amino acid content delivered to the tissue bed. Arterial concentrations are constant throughout the entire arterial system; therefore, any artery or sometimes an arterialized hand vein can be adequate for determining circulating amino acid concentration.⁵⁶ Blood

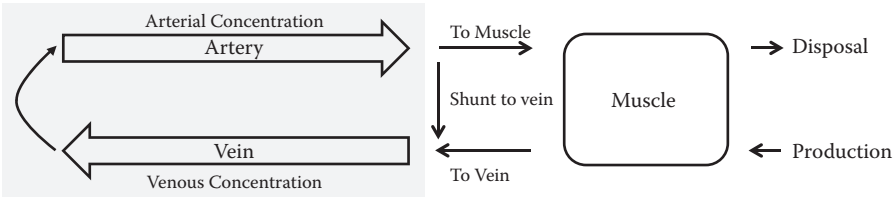


FIGURE 9.5 The three-pool model adds more information about protein kinetics than does the two-pool model (shaded region). Infusion of an essential amino acid tracer that is not metabolized within the muscle will cause production to be zero and allow disposal to be only protein synthesis.

flow must be accurately determined for A-V difference to yield reliable tissue balance results.^{57,58} The units of blood flow (~100 mL/min at rest per leg) are much larger than circulating amino acid concentrations (~50 nmol/mL), so a small error in blood flow measurements will have a large effect on the calculated amino acid delivered to the tissue. The choice of vein is critical and must be located downstream of the tissue of interest. Since veins drain blood from all tissues, the venous measurements are affected by all tissues (skeletal muscle, skin, bone, etc.) and are limited to the extent that the tissues contribute to amino acid metabolism.

In addition to A-V differences of amino acid, an isotopic tracer can be infused into a separate vein (commonly antecubital) to calculate amino acid R_a and R_d . R_a is the rate of appearance of amino acids into circulation from the tissue (represents protein breakdown) and R_d is the rate of disappearance of amino acids into the tissue (represents protein synthesis). The choice of tracer is crucial because the calculated R_a and R_d will reflect total tissue flux, including synthesis of nonessential amino acids. If a nonessential amino acid tracer is used, then R_a is affected by protein breakdown and *de novo* amino acid synthesis. Similarly, R_d provides an indication of amino acid uptake into tissue but cannot differentiate between nonoxidative fates of amino acids (such as gluconeogenesis), oxidation of the amino acid (such as for leucine), or protein synthesis. Thus, R_d for a leucine tracer includes protein synthesis and leucine oxidation, but R_d for phenylalanine (which is not metabolized in skeletal muscle) primarily indicates protein synthesis. An additional limitation is that amino acids may be released into the intracellular fluid and recycled for protein synthesis without entering general circulation. The two-pool model therefore underestimates protein synthesis depending on the degree of amino acid recycling. Thus, leg balance using a two-pool model yields values for R_a and R_d that *reflect* protein breakdown and synthesis but are not direct measures.

The three-pool model adds to the two-pool model by including the intracellular compartment of amino acids. Biolo et al. developed the model in order to evaluate amino acid transport kinetics into and out of the intracellular fluid.⁵⁹ The experimental design uses a continuous infusion of stable isotopes with samples from arterial and venous blood and muscle tissue. Amino acid transport rates can be determined from the artery to the muscle and from the muscle to the vein (Figure 9.5). The model also distinguishes the flow of amino acids from artery to vein that is shunted past the tissue. Rates of protein synthesis and breakdown are calculated from the net balance of amino acids and flow of blood into the muscle divided by the intracellular tracer enrichment.

The addition of amino acid transport kinetics to calculations of protein synthesis and breakdown are an important addition by the three-pool model. For example, Biolo et al. showed that a bout of resistance exercise increased the rates of inward transport for leucine, lysine, and alanine but not phenylalanine.⁶⁰ Since the model calculates both protein synthesis and breakdown, the study also identified that protein synthesis was elevated along with protein breakdown and indicated that both processes are affected by resistance exercise. A limitation of the three-pool model is that any limitations on amino acid transport or diffusion kinetics is unknown and could affect the intracellular concentration of amino acids. Previous work showed that the diffusion of glucose through interstitial fluid was a rate-limiting process for

glucose uptake into muscle cells and identified the possibility that amino acid transport may also be limiting.⁶¹ Microdialysis techniques can be used to add a fourth pool to the model and distinguish transport kinetics across membranes.⁶²

9.4.1.2.4 *Labeled Water*

It is possible to measure synthesis rates of proteins using a stable isotope of water called deuterium oxide (D_2O , “heavy water”). Low amounts of D_2O are normally found within the body water pool, but the method raises the enrichment to 2–3%. The body water pool undergoes proton exchange reactions that enrich the intracellular amino acid pool, which will ultimately be incorporated into proteins.⁶³ For human studies, D_2O is consumed in small volumes with an initial loading phase of 150 mL/day for 1 week followed by 100 mL/day for many weeks. Rodent studies use an initial intraperitoneal injection of D_2O followed by *ad libitum* access to an enriched water supply.⁶⁴ Tissue samples are collected before and after the study protocol and are analyzed for deuterium enrichment. Since the body water pool is used for many exchange reactions, a variety of end products can be measured during a single study design.⁶⁵ For example, muscle biopsies can be used to determine skeletal muscle protein synthesis rates while blood samples can yield cholesterol synthesis rates⁶⁶ or DNA synthesis rates.⁶⁷ The method allows synthesis measurements to be determined over long periods of times (weeks) as opposed to acute isotope infusion studies (hours). Although the method is well suited for long-term studies, it has recently been reported that D_2O can be injected intraperitoneally into animals as a flooding dose method.⁶⁴ A benefit is that protein synthesis can be calculated without the confounding effects of a flooding dose of amino acids. Short-term studies are helpful to understand acute effects in a well-controlled setting; however, long-term studies can evaluate if those acute effects will continue over the longer periods of time under less-controlled conditions. The use of deuterium oxide is not novel; however, it has regained popularity as its application to human health and nutrition is being explored. As described in 1935 by Schoenheimer and Rittenburg, “The number of possibilities of this method appears to be almost unlimited.”⁶⁸

9.4.1.2.5 *Components of Skeletal Muscle*

The term *skeletal muscle* is commonly used to distinguish it from smooth and cardiac muscle tissue. In reality, however, a tissue sample contains a wide variety of cell types, including contractile, structural, and circulatory elements. The analysis of muscle samples begins with homogenizing the entire sample and the calculated synthesis or breakdown rates are a combination of all proteins of the sample (termed mixed muscle protein synthesis [MPS] or breakdown). Protein breakdown rates reflect mixed muscle protein because they involve either tracer dilution (which cannot distinguish between proteolysis of different proteins) or release of bound tracer (requiring adequate incorporation of tracer). Release of 3-methyl-histidine attempts to quantify myofibrillar protein breakdown but is influenced by other protein sources as well.^{46,69}

Protein synthesis rates, however, can be distinguished between subfractions of muscle proteins by separation techniques. Improvements in the sensitivity of mass spectrometers allow analysis of these smaller muscle subfractions. Mixed muscle

samples are readily separated using differential centrifugation techniques into myofibrillar, sarcoplasmic, and mitochondrial subfractions. The separation of subfractions allows more specific analysis of muscle samples. The calculated FSR is determined by tracer incorporation into protein products and is influenced by the size and turnover of the protein pool. The large size of the myofibrillar protein pool will primarily determine MPS rates. However, separation of smaller pools such as mitochondrial proteins reveals higher FSR, indicating that mitochondrial proteins turn over at a higher rate than myofibrillar proteins.⁷⁰ Such analysis of subfractions yields important information regarding the response to different conditions including aging,⁷⁰ exercise,⁴⁸ and feeding.⁴⁷ The extracellular matrix is also a protein pool within mixed muscle that can respond to exercise stimuli along with myofibrillar and sarcoplasmic subfractions.⁷¹ More sophisticated separation techniques identified the FSR of individual muscle proteins and showed variable rates within subfractions.²³

9.4.2 CLINICAL EVALUATION OF PROTEIN STATUS

9.4.2.1 Dietary Records

Like most macronutrients and micronutrients, protein status can be determined by assessing dietary intake by a food record. There are a variety of ways to complete a food record and each has its advantages and disadvantages. The 3- or 7-day food record has the athlete record all food consumed in a 3- or 7-day period. It is usually recommended that the 3-day record include one weekend day. Both methods are relatively accurate (as compared to other methods), and this accuracy can improve with provision of a food scale. The technique works best if the athlete (or supervising dietician) records all food as it is consumed. Additional accuracy comes from weighing the food before and after consumption (such as an apple followed by its remaining core). However, left to their own devices, most athletes tend to underreport the amount of food consumed and the accuracy of reporting tends to decrease as the period of time extends (out to 7 days). Even though the 3- and 7-day records have their drawbacks, they tend to be more accurate than retrospective methods that require an athlete to recall all that he or she ate in the past 24 hours (24-hour recall) or interviews about the frequency of certain consumed foods (food frequency), or a combination in which a diet history is constructed from questionnaires. Nonetheless, there are now a variety of dietary intake software programs that can help determine protein intake over a period of time to determine the regular habits of that athlete.

9.4.2.2 Body Mass and Body Composition

A simple but gross measurement of protein status is body mass. Over a period of time, an athlete may gain or lose weight and this can easily be assessed by body mass. Since lean body mass makes up the majority of total body mass, one can assume that changes in body mass are associated with an increase or decrease of protein tissue. A better means to assess protein mass is the addition of some measurement of body composition. There are multiple ways to assess body composition, including underwater weighing, dual x-ray absorptiometry (DEXA), and electrical impedance. By knowing total mass and the composition of that mass, one

can calculate absolute lean body mass, which will roughly equate to the protein composition of the body. For example, an increase in lean body mass, even in the absence of an increase in total body mass, indicates a net accretion of protein. It is important to realize, however, that these are gross assessments and do not have the sensitivity to distinguish relatively small changes in protein status over a short period of time.

9.5 PROTEIN TURNOVER AND EXERCISE

Exercise training causes significant remodeling of skeletal muscle and produces training adaptations such as increased muscular strength, power, or endurance. The training adaptations and phenotypic changes that occur in skeletal muscle are due to the type of training stimulus placed on the muscles. For example, an endurance athlete can use low-resistance but high-repetition movements, such as running or cycling, to gain muscular endurance compared to an Olympic power lifter who uses high-intensity but low-repetition movements to gain muscular strength and power. The phenotypic changes reflect the type of exercise training and the molecular response of the muscle fibers to the training. Thus, the broad term of “exercise” should be clarified to specify resistance or endurance exercise, and the specific response of the muscle to each of these different stimuli will be discussed.

9.5.1 PROTEIN TURNOVER AND RESISTANCE EXERCISE

Chronic resistance exercise training using high-resistance and low-repetition movements, commonly using machines or free weights causes increases in strength and cross-sectional areas. The primary long-term adaptive response to resistance training is increased muscle strength from increased myofibrillar protein content in addition to other adaptations including connective tissue and neuromuscular adaptations.⁷² Under resting and fasted conditions, FBR is greater than FSR, indicating net catabolism.²² During a bout of strength training, it has been demonstrated that FSR is depressed.⁷³ In the period after strength training, FSR is increased⁷⁴ and can remain elevated up to 48 hours after the bout of exercise.²² In the absence of nutrient provision, FBR can still exceed FSR for a portion of the period.²² Importantly the increase of both FSR and FBR indicates more protein turnover and remodeling.

9.5.2 PROTEIN TURNOVER AND ENDURANCE EXERCISE

Endurance exercise has repeated repetitions of a relatively light load and generally does not lead to an enlargement in muscle. A lack of hypertrophy certainly does not mean that there is a lack of increase in protein turnover since there is substantial remodeling of the tissue to better use aerobic-derived energy sources and to resist fatigue. Increased mitochondrial protein content is well known with endurance training, providing a general indication that endurance exercise alters protein turnover. Studies in rats indicate that muscle protein synthesis is decreased during a bout of endurance exercise.⁷⁵ Unlike protein synthesis, the rate of whole-body protein breakdown increases during aerobic exercise.⁷⁶ After a bout of endurance exercise, rates of muscle protein synthesis

increase similarly to those found following resistance exercise.^{71,77,78} A large contribution to these increases is that of mitochondrial proteins, which show higher FSR than myofibrillar subfractions.⁴⁸ It is worth restating that in the absence of muscle hypertrophy, significant turnover of muscle protein occurs following endurance exercise.

9.5.3 TIMING OF PROTEIN INTAKE

Protein must be consumed in order to supply the body's amino acid pool over prolonged periods. Endogenous sources can provide amino acids; however, these may not be sufficient to promote training adaptations. As discussed previously, exercise activates a variety of pathways to promote protein turnover. This postexercise metabolic environment requires amino acids to allow adaptations. Feeding provides the necessary amino acid and energy substrates to promote recovery. The timing of nutrient intake around exercise is an important consideration for nutritional recommendation.

Under resting and fasting conditions, muscle protein breakdown is elevated in order to replenish the free amino acid pool and causes a net catabolism of skeletal muscle. Eating a mixed meal increases the availability of amino acids and promotes protein translation in a dose-responsive fashion.⁷⁹ However, the increase in protein synthesis is not indefinite and subsides over time.⁴⁷

Although exercise, at times, results in catabolic conditions, the provision of amino acids during or after exercise ensures an overall anabolic environment. Ingestion of amino acids after resistance exercise, either in isolated form⁸⁰ or as protein,⁸¹ stimulates the rate of muscle protein synthesis more than exercise alone.⁸² Further, it has been found that amino acid infusion after resistance exercise prevented the normal postexercise rise in muscle protein breakdown.^{80,83} In regards to endurance exercise, a mixed protein and carbohydrate beverage causes less negative whole-body protein balance during exercise and in the postexercise period as compared to carbohydrate alone.⁸⁴ More recently, it was found that consuming a combination of protein and carbohydrate after 2 hours of cycling resulted in a higher rate of mixed muscle FSR than consuming carbohydrate alone and that whole-body protein balance was positive only in the protein-plus-carbohydrate condition.⁸⁵ Thus, the addition of protein to carbohydrate as postexercise nutrition promotes muscle anabolism more than carbohydrates or fasting.

The timing of postexercise nutrition may affect its efficacy. In older humans performing 12 weeks of resistance exercise training, the consumption of a protein-containing supplement immediately following each bout of exercise resulted in greater increases in muscle strength and mass compared to delaying consumption by 2 hours.⁸⁶ Others found a greater stimulatory effect on muscle protein synthesis when a carbohydrate and essential amino acid mixture was provided before exercise than afterwards.⁸⁷ However, consumption of whey protein (as opposed to amino acids) did not have a differential effect of timing consumption before or after exercise.⁸⁸ The whey provided whole proteins instead of free amino acids; thus it appears that both the timing and form of amino acids can influence the recovery of muscle protein turnover following exercise.

A summary of nutrition and exercise indicates that feeding promotes storage of amino acids and exercise promotes adaptive remodeling, and the combination of

nutrition and exercise stimulates maximal adaptation.¹ Although simplistic, the summary is supported by nutrition and exercise effects on protein metabolism. For comparison purposes, the condition of 12-hour fasted values will be used as a baseline. Measurements of protein synthesis and breakdown after a 12-hour fast indicate that breakdown exceeds synthesis, so net protein balance is negative. If one is fed or receives an infusion of mixed amino acids (AA) after a fasted period, protein synthesis increases, whereas protein breakdown remains the same or decreases slightly. This response is indicative of a storage phenomenon in which synthesis increases without an increase in breakdown. In the period after exercise without nutrient provision, protein synthesis and protein breakdown are increased compared with the 12-hour fasted reference values, indicating that there is a stimulus (exercise) and remodeling (increase in synthesis and breakdown) response, although net balance does not improve to a positive balance. When there is an exercise stimulus with postexercise AA feeding, protein synthesis increases more than that after exercise or AA feeding alone, and protein breakdown remains similar to exercise without feeding. Because there is an increase in protein synthesis above the rate observed after exercise without AA provision, it is apparent that the provision of AA enhances protein synthesis. In addition, although protein breakdown is increased, it does not increase more than the fasted exercise response. Therefore, the increase in protein synthesis after feeding is a transient storage phenomenon, whereas physical exercise stimulates a longer-term adaptive response. Providing nutrition after physical activity takes advantage of the anabolic signaling pathways that physical activity has initiated by providing energy and AA building blocks for protein synthesis.

9.6 PROTEIN REQUIREMENTS

A full discussion of protein requirements is beyond the scope of this chapter. There is currently much discussion on the proper methods to determine protein requirements, although nitrogen balance has traditionally been used.⁸⁹ Further, these recommendations are limited because they assume energy balance and do not consider the potential role of the timing of protein nutrition on net protein balance. The current dietary reference intake (DRI) issued by the Institute of Medicine (IOM) is 0.8 grams of protein per kilogram body weight⁹⁰ (g/kg bw) and is consistent with the WHO recommendation.⁴ The recommendation is designed to meet protein requirements of 97.5% of the population and varies from the estimated average requirement (EAR) of 0.66 g/kg bw that covers 50% of the population. Importantly, these values were largely derived from a meta-analysis of studies using 235 individuals.⁹¹ It, of course, also begs the question whether athletes, both strength and endurance, fit within the recommendations for the general population.

It can be speculated that the increased protein turnover brought about by physical activity, whether increased amino acid oxidation or increased protein breakdown, may increase protein needs in athletes. The vast preponderance of anecdotal and recorded data^{92,93} indicates that athletes already consume protein far in excess of current recommendations. However, early studies indicated that instead of exercise raising protein requirements, exercise might lower protein requirements through protein sparing mechanisms.^{10,33} These initial studies are supported by others that

are not limited to the nitrogen balance technique.⁹⁴ The current consensus is that an intake of 1.1 gm/kg day in endurance athletes⁹⁵ and 1.3 g/kg bw in strength athletes⁹² is more than adequate to meet the needs of these athletes. Accordingly, a 70-kilogram athlete would require 77 to 91 gm of protein each day, which is readily consumed in Western culture. Additional protein intake may not induce additional benefits for skeletal muscle anabolism. For example, the increased skeletal muscle FSR following resistance exercise and 20 grams of protein intake was not different if protein was increased to 40 grams, suggesting that a maximal FSR was reached with 20 grams without additional stimulation from more protein.⁹⁶ Three issues, however, remain to be solved. First, does increasing protein above requirements increase sport performance? Second, does increasing protein intake during caloric restriction help retain lean body mass? And third, does the timing of protein nutrition after exercise ultimately change required habitual protein intake?

9.7 SPECIAL CONSIDERATIONS

9.7.1 SEX DIFFERENCES

Despite women having a smaller muscle mass than men,⁹⁷ attempts to detect sex differences in protein metabolism have produced little evidence of their existence. There have been some reports of differences between sexes in the rate of leucine oxidation at rest and during exercise,^{98–100} but there is no convincing evidence of major differences in whole-body protein turnover and mixed muscle protein synthesis^{76,98–103} between sexes even after accounting for different sizes of fat-free mass.^{98,101} Furthermore, it is reported that men and women do not have different rates of muscle protein synthesis in response to exercise.¹⁰² The lower protein mass in women may therefore be the result of an accumulated sex-specific hormonal effect on synthesis or breakdown over a period of many years.¹⁰⁴ Additionally, female sex hormones may influence protein synthesis muscle subfractions, which are not distinguished with measurements of mixed muscle in the whole body. For muscle collagen this does not seem to be the case,¹⁰³ but it could be for other protein fractions such as sarcolemmal enzymes or mitochondria. However, a more recent study has countered the argument that women have a higher decreased protein turnover than men.¹⁰⁵ The study was completed on thirty men and thirty-two women, thus substantially increasing power over previous studies. The results showed that women, irrespective of their body mass index and age, had higher FSR of muscle proteins and higher whole-body protein turnover than men. Since women have a smaller muscle mass than men, the increased FSR in women could be countered with increased FBR and cause no net anabolism. The issue of differences in protein turnover between sexes does not yet appear to be resolved, but at this point there is no reason to believe that one group, either men or women, has different requirements.

9.7.2 AGING

Whether older adults require protein in excess of the RDA is a contentious topic. With aging there is a progressive decline of skeletal muscle mass due to a variety of

causes. There is no doubt that there is a net catabolism in human muscle over time, yet current recommendations by the IOM for protein intake in older individuals are the same as recommended for all individuals over 19 years old.⁹⁰

It has been reported that whole-body protein synthesis declines with age even after adjustment for fat-free mass.¹⁰⁶ The same group used a large cohort of subjects to study changes throughout the lifespan and found a decline of 3–4% per decade in measures of whole-body protein turnover, breakdown, and synthesis.¹⁰² These studies are supported by others that indicated a decrease in muscle protein synthesis with age.^{74,102,107,108} In contrast, others have not found differences in muscle protein synthesis of younger and older individuals at rest.^{26,109–111} Still others proposed that the muscle wasting associated with aging is due to a decreased anabolic effect of feeding in older individuals.^{110,112,113} It is possible that muscle loss with age is a collective result of a decline in exercise habits along with decreased anabolic effects of nutrition.

The question then remains, do older individuals require more protein in their diet? Recently a large, well-controlled study using forty-two young and old individuals examined nitrogen balance during low, medium, and high levels of protein intake.¹¹⁴ The results indicated that nitrogen balance at the different protein intakes did not differ between younger and older subjects and that the current recommendation of 0.8 gm/kg body weight per day was sufficient to maintain nitrogen balance in older individuals. These data were quite convincing in that it was a very well-controlled study. However, as discussed, the appropriateness of nitrogen balance for protein adequacy continues to be debated. Therefore, the diversity of opinions on the adequacy of current recommendations for older individuals illustrates that the issue is not yet resolved.^{115–117}

9.8 FUTURE DIRECTIONS

There is still some discussion regarding adequate versus optimal protein metabolism. For example, can one protein intake be adequate but still fall short of additional benefits if more was consumed? This concept gets into the very core of the athlete psyche that more is better. To date, in a variety of athletic and special populations, there has not been convincing data presented that chronically higher intakes (irrespective of timing of intake) results in more optimal outcomes. However, there are still issues that need to be resolved regarding the quantity of protein required for different populations. Next, it is difficult to determine what the appropriate intake of protein is without the correct assessment measures. Nitrogen balance is the basis of all current protein recommendations but has great limitations. As of right now, no one has proposed a better alternative. Blunt clinical measures such as body composition are a way for athletes to assess their protein status but are fairly limited over the short term or for precise measures. Laboratory methods such as stable isotopes are capable of measuring much smaller changes in protein status but are still limited to short time frames. The isotope method using labeled water shows great promise in this area because of its ability to make long-term measures in free-living conditions (analogous to doubly-labeled water and energy expenditure). Finally, future studies must continue to account for the role of energy balance in protein metabo-

lism. Protein status cannot be assessed without consideration of energy balance since protein turnover is tied to energetic state.

9.9 CONCLUSIONS

Although not a large contributor to energy production, dietary protein intake is critically important for exercise-induced adaptations. Cells adapt to training with a response designed to make the body better at performing that stimulus later. The adaptation requires amino acid building blocks (from dietary proteins) to execute the making of the appropriate proteins to improve function in the cell. At the same time, proteins that are no longer necessary are removed because they are energetically costly to maintain. In the healthy state, it is clear then that protein structures within cells are always turning over protein for the benefit of the individual. Although some consensus has been reached that athletes do not require additional protein in their diet, there is a vast amount of work yet to be performed with consideration of how to optimize function or whether different subgroups (by sex or age) of athletes require different needs. To properly determine protein needs, there needs to be continued development of methods and techniques to assess protein status.

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10 Assessment of Vitamin Status of Athletes

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10.1 INTRODUCTION

Vitamins are organic compounds required in small amounts not only for survival but also necessary for athletic competitions. Vitamins cannot be synthesized in large enough quantities endogenously to meet metabolic requirements for efficient daily functioning and therefore must be consumed.^{1,2} Some vitamins must be converted to an active form or be incorporated into coenzymes, while some are capable of functioning without modifications.²

The vitamin status of athletes is often assumed based on outcomes of estimates of dietary intake (for example, diet recall and diet records). Given the metabolic demands of athletes, especially athletes training and performing in events that rely heavily on bioenergetic pathways, and the limitations of nutrition databases, it may be an unfortunate speculation to base nutritional recommendations on guidelines geared toward the general population. Conversely, those same athletes will not likely experience vitamin deficiencies if consuming a balanced and varied diet to meet their higher energy demands. For detailed insight into vitamin functions, adequate intake values, and food sources, other resources are available.² Also, the assumption of vitamin status from diet records or recall does not always account for differences in vitamin bioavailability and/or activity. The efficiency of vitamin absorption needs to be considered, as well as the interaction of the vitamin with other nutrients or

compounds that may increase or decrease availability. In addition to those physiological issues, some foods either may not be in a food database or have not been analyzed for complete vitamin content. Moreover, food processing is known to affect vitamin concentrations, which likely alters bioavailability.

However, given the temporal and financial costs associated with many biochemical assessments, not to mention the means of collecting tissue samples from athletes and the different metabolic demands of various sports, the vitamin status of and requirements for athletes are not fully known. With increasing interest in nutritional supplements and functional foods, the area of micronutrient analysis will likely expand to better determine their mechanisms of action. Therefore, the purpose of this chapter is to convey vitamin assessment strategies for those wanting to better determine vitamin status in athletes or to perform exercise interventions. Suggestions for the appropriate biological sample type (blood, tissue, and/or urine) will be discussed for those vitamins that potentially yield differences in concluding vitamin status. It must be noted that few studies have examined the influence of acute or chronic exercise on values of specific vitamins using human subjects. Therefore, caution and an open mind must be used when applying the following information.

In general, two critical issues to bear in mind when analyzing samples for vitamin content are: (1) utilize an acceptable vitamin extraction procedure and (2) ensure that appropriate measures or precautions are made to minimize the deactivation of the vitamins being measured. For example, when assessing biochemical vitamin status there are many factors that may influence the analysis, such as vitamin stability (see [Table 10.1](#)). If these influencing factors are not accounted for or controlled, then the results will likely be compromised.

10.2 ANALYTICAL CONSIDERATIONS

10.2.1 STABILITY OF VITAMINS

A critical methodological issue that occurs with the analysis of vitamins is their instability under certain conditions (exposure to light, oxygen, metals, etc.; see [Table 10.1](#)). This instability leads to changes in the structure (inactive form) and may ultimately yield spurious results. Therefore, it is critical to account for as many potential sample contaminants as possible.³ For example, when vitamin D is exposed to light, it will convert to isotachysterol and the 5,6-*trans*-isomer. As noted in [Table 10.2](#) and in the summary of assessments, most individual and multianalyte vitamin assessments require specific preparations and procedures; therefore, *post hoc* vitamin assessments are generally difficult to perform adequately.

10.2.2 VITAMIN BIOAVAILABILITY, ACTIVE FORMS, AND STORAGE

Basing vitamin status solely on nutrient intake values may likely yield inaccurate estimations of vitamin status, which may ultimately lead to either a vitamin deficiency or toxicity. This occurrence results from interactions that micronutrients and other dietary consumables have on the absorption and availability of other nutrients. The assessment of an athlete's diet may generate a different picture of vitamin status than

TABLE 10.1

Factors Responsible for Degrading or Inactivating Fat-Soluble and Some Water-Soluble Vitamins (Free Forms in Solution) That Can Influence Analysis Results

Influencing Factors	Vitamins													Prevention
	A	D	E	K	B ₁	B ₂	B ₆	B ₁₂	C	Niacin	Biotin	Folate ^a	Pantothenic Acid	
Air (O ₂) ^b	•	•	•		•			•	•			•		Exclude oxygen or air by replacement of inert gas
Heat	•	•	•		•	•	•		•		•	•	•	Work at lowest temperatures possible, store below –20°C (preferably –70°C)
Light, UV	•	•	•	•	•	•	•	•	•		•	•		Avoid sunlight (UV) and dim lights
Metals/Minerals	•		•					•	•			•		Avoid adding metals
Acid	•	•		•				•				•	•	Use acid-free solvents
Alkali		•	•	•	•	•	•	•	•		•	•	•	
Water		•												
Reducing Agents				•										
Oxidizing Agents	•	•			•			•						
Antioxidant	+	+	+	+								+		
Other		Iodine			Sulfite							Sulfurous acid, nitrite		
References	11, 33, 34	11, 33–35	11, 33, 34	11, 33, 34	1, 11, 20, 34, 35	1, 11, 34	1, 11, 34, 35	1, 11, 15, 34, 35	1, 6, 11	11	1, 34, 35	1, 11, 15, 34	1, 34	11, 33, 34

^a Folate exists in various forms and each form is sensitive to different factors.

^b Try to avoid exposure to air or oxygen during sampling or analysis; the vitamins may be more stable in the absence of oxygen, and this may minimize the sensitivity to these other factors. (Data from Lambert, W.E., Nelis, H.J., De Ruyter, M.G.M., and De Leenheer, A.P., in *Modern Chromatographic Analysis of the Vitamins*, De Leenheer A.P., Lambert, W.E., and De Ruyter, M.G.M., Eds., Marcel Dekker, New York, 1985, p. 1.)

Note: Add an antioxidant like butylated hydroxytoluene (BHT), α tocopherol, Vitamin C, propylgallate to samples prior to analysis to protect from oxidation. (Data from Ball, G.F.M., *Water-Soluble Vitamin Assays in Human Nutrition*, Chapman and Hall, London, 1994; Lambert, W.E., Nelis, H.J., De Ruyter, M.G.M., and De Leenheer, A.P., in *Modern Chromatographic Analysis of the Vitamins*, De Leenheer A.P., Lambert, W.E., and De Ruyter, M.G.M., Eds., Marcel Dekker, New York, 1985, p. 1.)

Note: Exposure of some vitamins to more than one of these factors can have an additive effect: more losses might occur when exposed to more than one factor. (Data from Lambert, W.E., Nelis, H.J., De Ruyter, M.G.M., and De Leenheer, A.P., in *Modern Chromatographic Analysis of the Vitamins*, De Leenheer A.P., Lambert, W.E., and De Ruyter, M.G.M., Eds., Marcel Dekker, New York, 1985, p. 1.)

TABLE 10.2
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment
Vitamin A		Colorimetry, spectrophotometry, fluorometry, capillary electrophoresis (Ref. 11); high-pressure liquid chromatography (HPLC), thin-layer chromatography (TLC) (Refs. 11, 34); gas chromatography (GC) (Ref. 7); and immunological or molecular biological techniques (Ref. 9)
	Blood	Serum β carotene levels (Ref. 2) Plasma retinal levels (only reduced if liver stores are depleted) (Ref. 2) Relative dose response (RDR) and modified relative dose response (MRDR) (Ref. 9); most representative of current status, and determine liver stores in a noninvasive way (Refs. 5, 9, 41) Retinol binding proteins (RBP) (Refs. 5, 9) Plasma retinol (generally accepted; see Ref. 9) and carotenoid analysis (Refs. 5, 42, 43); plasma retinol need not be adjusted for lipoprotein concentration (Ref. 18)
	Liver	Liver retinol levels (Ref. 44) (not practical; see Ref. 9)
	Tear fluid	Conjunctival impression cytology (CIC) (Ref. 9); least representative of current status (see Refs. 5, 9) Tear analysis (Ref. 5)
	Other	Dark adaptation test or night-blindness determination (Refs. 5, 8)
Vitamin D		HPLC, TLC, colorimetry, radioimmunoassay, gas chromatography-mass spectrometry (GC-MS) (Ref. 34); ligand-binding (Ref. 11)
	Blood	Plasma/serum vitamin D levels (Refs. 2, 10); need not be adjusted for lipoprotein lipid content (Ref. 18) Serum 25-OH-vitamin D levels is the sum of diet intake and production from sun exposure (Ref. 10)—the most valuable determinant of vitamin D status (Refs. 10, 11) Specific competitive protein binding assay for determination of 25-OH-vitamin D and 1–25(OH) ₂ vitamin D (of little value) (Refs. 10, 11) Radio receptor assay, using ³ H metabolites is a specific way to determine 1–25(OH) ₂ vitamin D (Ref. 11); also of little value (see Ref. 10) Plasma alkaline phosphatase activity (Refs. 2, 11)
	Tissue	Blood and tissue vitamin D metabolites are acceptable status indicators but are nonspecific (Ref. 11)
	Other	Indirect determination: serum calcium levels (Ref. 2) Clinical trial of supplementation (Ref. 5)
		Colorimetry, spectrophotometry, spectrofluorometry, TLC, HPLC, and GC (Ref. 11, 14)
Vitamin E (α TP)	Blood	Serum α TP (Ref. 14) in relation to serum triglyceride levels (Ref. 5)

TABLE 10.2 (continued)
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment
	Adipose	Platelet α TP levels in relation to serum triglyceride levels (Ref. 5)
		Plasma α TP is not a good indicator for toxic levels, because it reaches a plateau (Ref. 17)
		Erythrocyte α TP levels (Ref. 14)
		Adipose α TP levels in relation to serum triglyceride levels (Ref. 5)
	Muscle	Adipose α TP levels increase linearly with dietary intake of vitamin E (Ref. 15). Assess long-term vitamin E status (Ref. 17)
		Muscle α TP levels (Ref. 14), in close metabolic equilibrium with plasma α TP (Ref. 45)
	Urine	Urinary excretion of vitamin E may indicate excessive vitamin E intakes (Ref. 16)
	Other	Urinary excretion of vitamin E metabolite α CEHC is not used to assess vitamin E status (Ref. 16)
		Erythrocyte hemolysis by peroxide (Ref. 2, 5) is inversely related to plasma α TP concentrations (Ref. 16)
		Breath ethane (Ref. 16) and pentane (Ref. 13), levels are lipid peroxidation markers, high levels show depleted stores (Ref. 17)
		Functional tests: Vitamin E status can be assessed by oxidative changes in lipids: (1) Erythrocyte malondialdehyde test (<i>in vitro</i>) by H_2O_2 exposure (Ref. 13) (2) Erythrocyte malondialdehyde test with thiobarbituric acid (Ref. 13)
Vitamin K	Recommendation	Evaluate vitamin E levels in conjunction with blood lipid levels because vitamin E is carried on lipid-protein complexes called lipoproteins. Evaluating blood vitamin E levels alone when assessing vitamin E status is misleading (Ref. 16) It is more useful to use more than one biochemical tests to assess vitamin E status (Ref. 13)
	Blood	HPLC, TLC, colorimetry, GC-MS (Refs. 34, 46)
		Plasma prothrombin concentrations (Refs. 2, 46)
		Plasma phylloquinone (Ref. 13) reflects phylloquinone intakes (Ref. 46) but does not correlate well with vitamin K status (Ref. 46)
		Plasma or serum des- γ -carboxyprothrombin (DCP); most sensitive indicator of vitamin K status (Ref. 5, 18, 22, 46) DCP:prothrombin ratios (Ref. 13)
	Urine	Direct chromatographic vitamin K assays (Ref. 47)
		Urinary γ -carboxyglutamic acid (Ref. 13)
	Other	Hydroxyapatite binding capacity of osteocalcin (Ref. 13)

(continued)

TABLE 10.2 (continued)
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment
Thiamin (B ₁)		Ratio of prothrombin activity to the total immunochemical equivalents of prothrombin (Ref. 46)
		Ratio of Simplastin thromboplastin activated prothrombin time to activated by <i>Echis carnatus</i> venom (S:E ratio) (Ref. 46)
		Bleeding and clotting time (Ref. 5)
		Prothrombin time (Ref. 5); still used but is insensitive and nonspecific as primary method to determine vitamin K status; Ref. 37)
		Measurements of uncarboxylated osteocalcin (Ref. 46)
	Recommendation	Use both the plasma prothrombin measurements and DCP (Ref. 13)
		Plasma or serum des- γ -carboxyprothrombin (DCP) and osteocalcin; most sensitive indicator of vitamin K status (Refs. 5, 18, 21, 46)
	Blood	HPLC (Refs. 5, 19, 34), TLC (Ref. 34), ion exchange chromatography (Ref. 48), colorimetry (Ref. 5), enzymatic (Refs. 5, 20, 22), and microbiological assays (Refs. 5, 34)
		ETKAC (erythrocyte transketolase activity) (Refs. 5, 19, 22); highly reliable method to determine status (Ref. 11)
		Serum thiamin levels (Refs. 5, 20, 21); insensitive status indicator (Ref. 11)
		Thiamin pyrophosphate (TPPE) test of erythrocytes (Refs. 20–22)
		Blood pyruvate, lactate, and α ketoglutarate levels (Ref. 21)
Riboflavin (B ₂)	Urine	Whole blood (<i>Lactobacillus viridescens</i> assay) (Ref. 47); insensitive status indicator (Ref. 11)
		Erythrocyte thiamin levels; insensitive status indicator (Ref. 11)
		Urinary excretions of thiamin (reflect thiamin status of the previous 24 h) (Refs. 2, 5)
		Urinary excretions of thiamin metabolites (Ref. 21)
		Microbiological assays (Refs. 5, 34)
	Body fluids	<i>Ex vivo</i> lymphocyte growth response (Ref. 5)
	Other	Cerebrospinal fluid (CSF) thiamin levels (Ref. 21)
	Recommendation	Use ETKAC and TPPE together to assess thiamin status for most reliable results (Ref. 21)
		Use more than one method or test to assess thiamin status (Ref. 49)
	Blood	HPLC (Refs. 5, 24, 34), TLC (Refs. 34, 50), fluorometry (Refs. 5, 23), enzymatic (Refs. 2, 5, 24), and microbiological assays (Refs. 5, 34)
		Erythrocyte glutathione reductase activity coefficient (EGRAC) (Refs. 2, 23, 24) is the most common and most sensitive to tissue stores (Refs. 23, 24)

TABLE 10.2 (continued)
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment	
Niacin	Urine	Blood riboflavin levels (Refs. 23) is an insensitive indicator of riboflavin status (Ref. 24)	
		Erythrocyte riboflavin levels (Ref. 23) is an insensitive indicator of riboflavin status (Ref. 24)	
		24 h collection of urinary excretions of riboflavin reflects dietary intake (Ref. 23) and is not a sensitive indicator for tissue stores (Ref. 23)	
		Urinary excretions collected (1) at random, (2) after fasting, (3) a 24 h specimen, and (4) after load return test (Ref. 23)	
	Body fluids	Ratio of urinary riboflavin levels to creatinine levels (Ref. 22)	
		Microbiological assays (Refs. 5, 34)	
	Other	<i>Ex vivo</i> lymphocyte growth response (Ref. 5)	
	Recommendation	EGRAC together with urinary excretion test (Refs. 11, 24)	
	Vitamin B ₆	Blood	HPLC (Ref. 33), chromatography (Ref. 25), colorimetry (Ref. 51), fluorometry (Ref. 25), enzymatic (Ref. 25), and microbiological assays (Ref. 2, 5, 34)
			Serum niacin level determination is not a sensitive test (Ref. 22); it reflects dietary intake, not tissue stores (Ref. 11)
Erythrocyte NAD levels (Ref. 5)			
Erythrocyte NAD:NADP ratio (Ref. 5) is used to evaluate niacin status (Ref. 25)			
Blood and tissue		Lowry method measures NAD and NADP by using specific dehydrogenase enzymes (Ref. 25)	
Urine		Excretion of N'-methylnicotinamide (NMN) and 2-pyridone (Ref. 5) after a tryptophan dose (Ref. 2); widely used method (Ref. 22)	
		Ratio of 2-pyridone to NMN is a recommended method (Ref. 24)	
Body fluids		Microbiological assays (Refs. 5, 51); <i>Lactobacillus plantarum</i> (Ref. 3)	
Other		<i>Ex vivo</i> lymphocyte growth response (Ref. 5)	
Vitamin B ₆		Blood	NMN excretion expressed as mmol/mol creatinine is not an accurate method (Ref. 22)
	Dowley-1-formate chromatography is used to separate the puridine nucleotides (NAD/H and NADP/H) and NMN (Ref. 25)		
	NMN assessment done after 4–5 h after a 50 mg load of nicotinamide (Ref. 24)		
	HPLC (Ref. 34), TLC (Ref. 33), GC (Ref. 52), enzymatic (5, 26), and microbiological assays (Ref. 5, 34)		
	Plasma pyridoxal phosphate (PLP) concentrations (Refs. 26, 53); most often used, and correlates with tissue stores (Ref. 11)		
		Plasma total vitamin B ₆ levels or plasma PL levels (Ref. 26)	
		(continued)	

(continued)

TABLE 10.2 (continued)
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment
Cobalamin (B ₁₂)	Urine	*Plasma homocysteine levels (Ref. 5) after a methionine load (Refs. 15, 22, 26) reflect hepatic vitamin B ₆ status (Ref. 26)
		Serum 4-PA levels (Ref. 5)
		Erythrocyte PLP levels is useful as an additional index (Ref. 26)
		*Erythrocyte ALT and AST activation coefficients (Refs. 5, 15, 23, 26, 54); reflect long-term vitamin B ₆ status because of the lifetime of the erythrocyte (Ref. 26)
		Erythrocyte α-EGOT measurements (Ref. 53)
		Urinary 4-PA excretion (Refs. 2, 15, 26), which is a short-term indicator (Ref. 26) that reflects dietary intake (Ref. 22)
		Urinary total vitamin B ₆ (Ref. 26)
		Urinary pyridoxal lactone (Ref. 15)
		*Urinary metabolite (xanthurenic and kynurenic acid) excretion (Ref. 5) after a tryptophan load (Refs. 11, 15, 22, 26); reflect hepatic vitamin B ₆ status (Ref. 26)
		*Urinary homocysteine levels (Ref. 5) after a methionine load (Refs. 15, 22, 26) reflect hepatic vitamin B ₆ status (Ref. 26)
	Body fluids	Urinary PL expressed as mg per g creatinine (Ref. 11)
	Other	Microbiological assays (Refs. 5, 34, 55)
	Recommendation	*Oxalate excretion (Ref. 26); less common method
		*EEG pattern (Ref. 26); less common method
		<i>Ex vivo</i> lymphocyte growth response (Ref. 5)
		Plasma or urine amino acid levels and ratios (Ref. 5)
Use at least two biochemical indices; one must be the PLP test.		
Plasma PLP and tryptophan load test together is an excellent biochemical confirmation of vitamin B ₆ status (Ref. 11)		
	(*) These are indirect methods that do not necessarily reflect total vitamin B ₆ in tissue or serum; they indirectly reflect PLP in certain tissue (Refs. 22, 26)	
Blood	Radioimmunoassay (Refs. 27, 34); microbiological assays, dual isotope methods (Ref. 5)	
	Serum cobalamin assay is a standard method (Refs. 5, 27)	
	Erythrocyte vitamin B ₁₂ measurement is a common biochemical test (Ref. 11)	
	Holo TC-II (vitamin B ₁₂ transporter) measurements detect early vitamin B ₁₂ deficiency (Refs. 11, 27)	
	Plasma total vitamin B ₁₂ levels (Ref. 22)	
	Plasma and urine	Measurement of substrates, methylmalonic acid (MMA), and homocysteine, of two vitamin B ₁₂ –dependent enzymes is a new and more accurate way of assessing intracellular deficiencies (Refs. 5, 27)
		Plasma MMA measurements are better than plasma homocysteine measurements (Ref. 27)

TABLE 10.2 (continued)
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment
Folate	Urine	Urinary total vitamin B ₁₂ levels (Ref. 22)
	Biological fluids	Microbiological assays (Refs. 5, 34)
	Other	Vitamin B ₁₂ deficiency is reflected by high levels of 2-methylcitrate, N,N-dimethylglycine, N-methylglycine, and cystathionine <i>Ex vivo</i> lymphocyte growth response (Ref. 5) Shillings test or dual isotope variations for vitamin B ₁₂ absorption (Ref. 5)
		HPLC (Ref. 34), radioimmunoassay (Ref. 34), radiometry (Ref. 28), fluorometry (Refs. 28, 56), TLC (Ref. 28), enzymatic assay (Ref. 28)
	Blood	Serum folic acid levels (Refs. 2, 5, 22); should not be used by itself (Ref. 11, 57) Erythrocyte folic acid levels (Ref. 2, 5, 22) Serum or erythrocyte THF by radio isotope assay (Ref. 28) Serum or erythrocyte 5-methyl THF (Ref. 28) Serum folate activity (Ref. 28) Erythrocyte folate status is a reliable indicator for long term of folate status (Ref. 22) and tissue stores (Refs. 11, 29, 57) Serum homocysteine concentration is an ancillary indicator of folate adequacy (Ref. 29)
	Urine	Urinary folate levels (Ref. 2) Urinary N-formimino glutamic acid indicates comprised folate stores (Ref. 15) indirectly but is not sensitive enough and not used frequently (Ref. 11)
	Biological fluids	Microbiological assays (Refs. 5, 34); <i>Lactobacillus rhamnosus</i> (Ref. 3)
	Other	<i>Ex vivo</i> lymphocyte growth response (Ref. 5) Neutrophil hypersegmentation (Refs. 5, 57) Dihydrofolate reductase (DHFR) inhibition assay (Ref. 56)
		HPLC (Ref. 29, 33), fluorescent assay (Ref. 30), TLC (Ref. 34), microbiological assay (Ref. 2, 5, 34), colorimetry (Ref. 5)
	Blood	Whole-blood biotin levels; not a sensitive indicator (Ref. 11, 30) Avidin-binding assay (Ref. 30, 58, 59) Derivatives of biotin (Ref. 30)
Biotin	Urine	Urinary biotin levels (Refs. 2, 5, 15) Urinary excretion of 3-hydroxyisovaleric acid (inversely related to biotin status) (Ref. 31) is a sensitive, early detector of biotin deficiencies (Refs. 11, 31)
	Biological fluids	Microbiological assays (Refs. 5, 34)
	Other	<i>Ex vivo</i> lymphocyte growth response (Ref. 5) Propionyl-CoA carboxylase and pyruvate carboxylase (biotin dependent enzymes) activity in hair roots (Ref. 60)

(continued)

TABLE 10.2 (continued)
Biochemical Indices to Assess Vitamin Status

Vitamin	Component	Biochemical Tests for Status Assessment
Pantothenic Acid	Recommendation	Chromatographic separation of biotin analogues together with avidin-binding assay (Ref. 30)
		HPLC (Ref. 34), colorimetry (Ref. 5), microbiological assay (Refs. 5, 34)
	Blood	Whole-blood pantothenic acid levels (not very sensitive) (Refs. 5, 11)
		Serum pantothenic acid (not very sensitive) (Ref. 11)
	Urine	Urinary pantothenic acid levels (not very sensitive) (Refs. 5, 11)
Vitamin C	Biological fluids	Microbiological assays (Refs. 1, 12), using yeast and lactobacillus for blood and urine pantothenic acid measurements (Refs. 2, 32)
	Other	<i>Ex vivo</i> lymphocyte growth response (Ref. 5)
		HPLC (Refs. 5, 61), TLC (Refs. 33, 61), GC (Ref. 61), spectrophotometry, fluorometry, chromatography, electrochemical techniques (Ref. 6)
	Blood	Serum ascorbate levels (Ref. 61); easy to perform, and most often used, also reliable (Refs. 2, 6)
		Leukocyte ascorbate levels, most reliable (Ref. 6); reflect tissue and blood ascorbate content and correlates with liver ascorbate (Refs. 6, 11, 16)
		Platelet ascorbate level (Ref. 6)
	Urine	Urinary ascorbate level measurement is not a good indicator of vitamin C status because vitamin C is reabsorbed by the kidneys (Ref. 6), but is a good indicator for current status (Ref. 11)
	Saliva	Salivary ascorbate level measurement is not a good indicator of vitamin C status (Ref. 6)
	Other	Oral loading test (Ref. 5)
		The automated and microtiter plate spectrophotometric method is used for measurement of plasma and leukocyte ascorbate; it is fast and has high sensitivity (Ref. 6)
		Whole-blood and red blood cell ascorbate measurements are less sensitive (Ref. 6)

what is derived from the analysis of biochemical samples. This conflict may go unnoticed if vitamin intake alone is assessed. This may be common in some instances, as the availability of computer-assisted nutrient analysis programs has increased, but the access to and costs of the numerous biochemical assessments to measure the concentration of each vitamin are likely limited for the majority of coaches and athletes. [Table 10.3](#) displays the tissues or sample sites that can be used for assessment, as well as the compound and the analytical method used to verify vitamin status.

This oversight may occur with the assessment of vitamin B₆. For example, two sources of dietary vitamin B₆ are pyridoxine (PN) and pyridoxine-5'-β-D-glucoside

TABLE 10.3**The Active Forms and Storage Sites for Vitamins**

Vitamin	Active Form(s)	Storage Site
A	(all- <i>trans</i>) Retinol (Refs. 2, 9) (all- <i>trans</i>) Retinal (Refs. 2, 9) Retinoic acid (Refs. 2, 9) B-carotene	Liver (retinol) (Refs. 2, 9, 33) Adipose tissue (carotenoids) (Ref. 8)
D	1,25(OH) ₂ D (Refs. 2, 36) 24,25(OH) ₂ D (Refs. 2)	Skin (7-dehydrocholesterol) (Ref. 36)
E	d- α -tocopherol (most active) (Refs. 2, 12) β -tocopherol (Ref. 2) γ -tocopherol (Ref. 2) δ -tocopherol (Ref. 2) Trienols (Ref. 2)	Adipose (adipocytes) (Refs. 2, 15, 17) Lipid fractions of membranes (Ref. 2) Adrenals, liver, and muscles (Ref. 2, 15)
K	Hydroquinone (Ref. 38)	Adrenal glands, lungs, bone marrow, kidneys, and lymph nodes (Ref. 13)
Thiamin (B₁)	Thiamin Pyrophosphate (TPP) (Refs. 2, 15)	Skeletal muscle, liver, heart, kidneys, and brain (Refs. 2, 21)
Riboflavin (B₂)	FMN, FAD (Refs. 2, 34)	Liver, heart, and kidneys (Refs. 2, 15)
Niacin	NAD, NADP (Ref. 2)	
Pyridoxine (B₆)	Pyridoxal phosphate (PP) (Ref. 2)	Muscle (Refs. 2, 26), liver (Ref. 2)
(Cyanocobalamin (B₁₂))	Methylcobalamin (Ref. 2), adenosylcobalamin (Ref. 2)	Liver (Ref. 26), brain, kidney, spleen, and muscle (Ref. 15)
Vitamin C	Reduced ascorbic acid (DHAA) (Ref. 2), ascorbic acid (AA) (Refs. 6, 39)	Pituitary and adrenal glands (Refs. 6, 15), leukocytes (Ref. 6), eye tissue (Ref. 16), less in saliva and plasma (Refs. 6, 16)
Biotin	Biocytin (Ref. 2)	Muscle, brain, and liver (Ref. 15)
Folate	Tetrahydrofolic acid (THF) (Ref. 2)	Small amounts in liver, cerebrospinal fluid, bone marrow, spleen, and kidney (Ref. 2)
Pantothenic acid	Portion of coenzyme A (Refs. 2, 40)	

(PN-glucoside). Both PN and PN-glucoside are converted to vitamin B₆; however, the conversion of PN-glucoside is less than that of PN, and PN-glucoside slightly inhibits the conversion of PN.⁴ Therefore, if an athlete ingests foods that contain higher levels of PN-glucoside, then the athlete's actual vitamin B₆ levels may not adequately reflect vitamin B₆ intake unless the nutrient assessment procedure accounts for the influence of PN-glucoside.

Another complication may arise when measuring serum or plasma levels of particular vitamins. The concentrations of vitamins in circulation may only reflect the current vitamin status. Whereas, if the storage forms (if applicable) of the vitamin were to be assessed, a deficiency may be evident. As with iron assessments, it can be

important to measure the stored vitamin concentration. That is, an individual may have a limited supply of stored vitamins for continued normal functioning, even though circulating levels appear in the normal range.

10.2.3 BIOCHEMICAL INDICES TO ASSESS VITAMIN STATUS

Determining vitamin status is dependent on the source of sample used for quantification. For some vitamins it may be more beneficial to measure stored levels versus amounts in circulation, as the concentrations in circulation will not reflect a deficiency until the stored levels have been sufficiently diminished. Contrarily, assessing certain samples may not be justifiable. For example, liver samples provide an accurate measurement of vitamin A (retinol) levels, but the necessary biopsy would be excessive as similar results can be obtained by measuring plasma retinol levels. Some have reported the assessment techniques using tear⁵ or saliva samples⁶ to assess vitamin status, but those minimally invasive techniques are generally less representative of the individual's vitamin status than results obtained from blood or tissue samples. Therefore, when assessing vitamin status, it is imperative to carefully select the sample source, preparatory methods, and analytical techniques. Otherwise, the time and money spent for this assessment may not yield optimum results. In other words, the assessment would not have performed up to its potential.

10.3 ASSESSMENT GUIDELINES AND CONSIDERATIONS

The following information was compiled to aid in deciding which indices and techniques to choose in order to assess the status of a particular vitamin. These guidelines are not all encompassing, as many of these procedures have not been used in conjunction with athletes. Additionally, other modifications or adjustments not mentioned here may need to be made given differences in analytical equipment and skill of personnel.

Vitamin A (Retinoids). Several techniques and laboratory equipment can be used to assess vitamin A status (Table 10.3). Different forms of vitamin A (β -carotene, retinol, or retinal) can be determined from blood, liver, or tear fluid. Carotenoid concentrations in blood and tissue samples usually reflect dietary intakes.⁷ Vitamin A status can also be determined indirectly by conducting a night-blindness test.^{5,8} The relative dose response and modified relative dose response method, where liver stores are assessed by giving the person an oral vitamin A dose, is most representative and noninvasive.⁹ The conjunctival impression cytology test is the analysis of vitamin A levels of cells taken from a person's eye but is less representative of current vitamin A status.⁹

Vitamin B₁ (Thiamin). Erythrocyte (red blood cell) transketolase is an enzyme that needs thiamin to function, and when thiamin is depleted, this enzyme's activity decreases. Therefore, by measuring the enzyme activity, vitamin B₁ status can be determined.²⁰ Thiamin is needed in the form of thiamin pyrophosphate (TPP).²¹ Erythrocyte transketolases activity can be measured by

adding TPP to the reactions. After TPP is added, increased activity would indicate thiamin deficiency. This suggests there was not enough thiamin for the enzyme to function before the addition of TPP.²² Other methods, such as serum thiamin, microbiological assays, and erythrocyte thiamin levels, are insensitive indicators of vitamin B₁ status (Table 10.3).

Vitamin B₂ (Riboflavin). Erythrocyte glutathione reductase activity coefficient is a test utilizing the activity of the enzyme erythrocyte glutathione reductase to determine vitamin B₂ status (Table 10.3).²³ The activity of the enzyme is measured before and after addition of flavin-adenine dinucleotide (FAD), a coenzyme needed for the enzyme to function.^{23,24} If the activity of the enzyme is low before and higher after the addition of FAD, that would suggest a vitamin deficiency. If there were no deficiency, the levels before and after FAD addition would be the same. Other methods, such as blood, red blood cell, and urinary riboflavin levels, are also used to assess vitamin B₂ levels, but are not very sensitive indicators.²³

Niacin (Nicotinamide and Nicotinic Acid). Niacin is a precursor for nicotinamide adenine dinucleotide (NAD), which is very important for biological functions.²⁵ Erythrocyte NAD levels as well as the ratio of erythrocyte NAD:NADP can be used to determine niacin status in blood.²⁵ The Lowry method has been developed, using specific dehydrogenase enzymes, to measure NAD and NADP levels in blood and tissue. Urinary excretions of niacin metabolites, such as N'-methylnicotinamide (NMN) and 2-pyridone, are also widely used methods in assessing niacin status (Table 10.3).²⁴

Vitamin B₆ (Pyridoxal). Table 10.3 displays methods by which vitamin B₆ can be assessed. Vitamin B₆ levels and vitamin B₆ metabolites in blood and urine samples are generally used for assessing this vitamin's status.^{5,26} Pyridoxal phosphate (PLP or PP) is the active form of vitamin B₆, and its levels in blood reflect tissue stores.^{2,11} Enzymatic tests, such as erythrocyte alanine transaminase (ALT) and erythrocyte aspartate transaminase (AST), indirectly reflect vitamin B₆ status.⁵ A short-term indicator for vitamin B₆ status is urinary 4-pyridoxic acid (4-PA), a metabolite, representing 40–60% of the daily intake of vitamin B₆.²⁶ Urinary total vitamin B₆ levels represents 8–10% of the daily intake.²⁶

Vitamin B₁₂ (Cobalamin). Vitamin B₁₂ status is generally assessed using blood samples,²² where total vitamin B₁₂ levels are assessed (Table 10.3). Enzymatic tests are also used where substrates, such as methylmalonic acid (MMA), holotranscobalamin (holoTC), and homocysteine, are measured. Vitamin B₁₂-dependent enzymes utilize these substrates. High substrate levels in blood or urine indicate that there is not enough vitamin B₁₂ for the enzymes to function, so they cannot use the substrates. Therefore high substrate levels indicate deficiency.^{5,27}

Vitamin C (Ascorbic and Hydroascorbic Acids). Leukocyte (white blood cell) vitamin C (ascorbic acid) concentration is the most reliable determinant of vitamin C status and is not affected by recent fluctuations in the diet (Table 10.3).^{6,11} It is representative of tissue and blood vitamin C status and is correlated with liver vitamin C stores.^{6,11,16} Serum and platelet vitamin C

levels can also be used to assess vitamin C status.⁶ Urinary levels are not a good indicator because the kidney reabsorbs vitamin C.⁶ Blood and urinary vitamin C levels also reflect recent dietary intake.¹¹

Vitamin D (Calciferol). Different forms of vitamin D exist in blood and tissue, such as 25-OH vitamin D and 1,25 (OH)₂ vitamin D (Table 10.3). In assessing vitamin D status of the body, it is most valuable to assess 25-OH vitamin D levels in blood. The reason for this is because 25-OH vitamin D is converted to 1,25 (OH)₂ vitamin D (active form, Table 10.2). In the blood, 25-OH vitamin D is generally present in much higher concentrations than 1,25 (OH)₂ vitamin D,¹⁰ and therefore it will always be converted to the active form, even though the active form appears in low, normal, or high concentrations. Other methods, such as assessing the proteins that carry vitamin A in the blood (retinol binding proteins), enzymatic tests, and receptor binding tests, are also used but are not as efficient as the method discussed earlier.¹¹

Vitamin E (Tocopherols and Tocotrienols). Alpha tocopherol (α TP) is the most active form of vitamin E in the body, and this form is assessed when determining vitamin E status.^{2,12} In Table 10.3, the different techniques to assess vitamin E status are listed, such as the use of colorimetry, thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC), and the value of different methods are mentioned. According to Groff and Gropper,¹³ there is not one method that is very accurate in determining vitamin E status; therefore, it is recommended to use more than one biochemical test. Blood, erythrocyte, adipose, muscle, and urine α TP levels have been used to determine vitamin E status.^{14–16} Vitamin E protects lipids in the body against oxidative damage (peroxidation). By exposing samples to oxidation, vitamin E status can be determined by the time it takes for these samples to be damaged. High vitamin E levels in the samples will extend or delay the damage, or such levels can be measured by lipid peroxidation markers like breath ethane and pentane.^{13,16,17} In the latter test, high levels of peroxidation markers show depleted vitamin E levels. Vitamin E is carried on lipid-protein complexes called lipoproteins in the blood and when assessing vitamin E, the lipids in these lipoproteins must be taken into account because assessment of blood vitamin E levels alone are misleading in determining vitamin E status.¹⁸

Vitamin K (Phylloquinone, Menaquinone, Menadione and Undercarboxylated Osteocalcin). By measuring blood prothrombin, phylloquinone, or des- γ -carboxyprothrombin (DCP) levels (Table 10.3), vitamin K status can be assessed. Of these three, determining DCP by utilizing antibodies is the most valuable indicator. Other samples, such as urine, can also be used to determine vitamin K status. The recommendation, however, is to use DCP as well as blood prothrombin determinations.¹³ Bleeding and clotting time of blood is also useful to determine vitamin K status.⁵ For the assessment of bone-related levels of vitamin K, measuring the percent of undercarboxylated osteocalcin is also performed.¹⁹

Folate (and Folacin). Folate (folic acid) is present in the body in the form of tetrahydrofolate, which is very important for many biological pathways in the body.²⁸ By measuring metabolites of these pathways, folate status can be determined (Table 10.3). An example is urinary N-formiminoglutamic acid. This metabolite is involved in the breakdown of histidine to glutamic acid and requires the cofactor tetrahydrofolate. Without tetrahydrofolate, levels of N-formiminoglutamic acid in the blood will increase and lead to excretion in the urine.¹⁵ Assessment of serum folic acid levels by itself should not be used for folate status determination.¹¹ Red blood cell folate levels represent tissue store, thus this measurement is a more reliable long-term indicator.^{22,29}

Biotin. A reduced biotin level in urine is a determinant of biotin deficiency.³⁰ Also, the metabolite 3-hydroxyisovaleric acid, which is excreted in urine, is inversely related to biotin status.³¹ It is also a sensitive and early indicator of biotin deficiency.^{11,31} Blood biotin levels are not sensitive indicators for biotin status, even though the avidin-binding assay or bioassays were used (Table 10.3).³⁰

Pantothenic Acid. Pantothenic acid is present in the body as part of acetyl coenzyme A (CoA), which plays a critical role in energy metabolism. After blood samples are obtained, certain enzymes, called hydrolytic enzymes, are needed to cleave pantothenic acid from CoA in order to be analyzed in the laboratory.³² This cleavage is not needed for determining pantothenate levels in the urine, because hydrolytic enzymes present in the body already cleaved pantothenate from CoA.³² Another means of determining blood, tissue, and urine pantothenate is by microbiological assays using yeast and lactobacillus (Table 10.3).²

Although chemical assessment of physical samples is a preferred means of determining vitamin status, this process can be too costly or cumbersome for some athletes or their coaching staff. Thus, many athletes and coaches attempt to assess vitamin status using indirect assessments, particularly diet records or diet recalls. This is evident especially among the scientific community, as most published reports pertaining to vitamin status in athletes have used indirect methods to estimate nutrition status of the population sample.

Indirect assessments provide useful information regarding what athletes from various sports eat and shed light on the amount of each vitamin those athletes ingest during the prescribed assessment period. For example, in a group of ultra-distance runners, it was reported this group had insufficient intakes of several antioxidant vitamins and that vitamin intake was associated with low energy intake.⁶² Interestingly, they observed that antioxidant status of the runners was actually similar to other runners, thereby illustrating the need to measure biological samples to substantial nutrition status instead of making assumptions based on what is eaten.

As a guide, using indirect measures of vitamin status can be useful for the water-soluble vitamins, but doing so for the fat-soluble vitamins may lead to false security or alarm. In other words, the indirect methods can be a useful tool to determine which vitamins may need to be analyzed directly. This screening could reduce costs

and time by focusing the assessments on vitamins that seem to be consumed at lower than recommended levels. Also, many of the recent papers that have been published regarding vitamin status have pertained almost exclusively to the antioxidant vitamins and not specifically the vitamin status.

10.4 ASSESSMENT METHODS

Absorption spectrophotometry is limited to vitamins with a strong chromophore. Light spectrophotometry utilizes either ultraviolet or visible wavelengths to measure the sample. As the selected wavelength of light passes through the sample, the absorption of light will vary according to the sample. Based on a standard curve or derived regression equation, the absorption is proportional to the vitamin content of the sample.

Capillary electrophoresis is a high-performance analytical technique that can be used to separate a variety of charged and neutral components. When voltage is applied through the run buffer, the particles present will migrate through a tube at a velocity determined by their respective size and electric charge. This separation technique is synonymous with other forms of electrophoresis.

Chromatography (gas, thin-layer, and paper) is a separation procedure similar to HPLC. It is capable of separating the desired compound but is not capable of quantifying the amount or concentration of the sample. For example, following gas chromatography, the sample is further assessed using mass spectroscopy to quantify the amount of the desired compound.

Diet analysis is the most common method for assessing vitamin status. This method indirectly assesses status. There are several means to analyze a diet (for example, diet recall and food frequency questionnaire), yet each method cannot definitively ascertain whether the athlete is truly deficient. Dietary analysis can be a useful tool to determine which vitamins might be deficient in an athlete. Hence this process may help narrow down the number of vitamins to analyze, which could significantly decrease analytical costs.

Electrochemical techniques are used following chromatography to quantify vitamin content. The procedure is based on the electrochemical qualities of each vitamin or vitamin.

Enzymatic and microbiological assays utilize chemical reagents to convert the vitamin or vitamin to a compound that is generally measurable by fluorometry or various types of spectrophotometry. Specific to microbiological assays, the sample is exposed to an organism that will only grow in the presence of the specific vitamin.

Fluorometry is similar to spectroscopy. The exception is that the fluorometer detects the fluorescence of compounds at different wavelengths. Therefore, if the assay reagent does not yield fluorescent compounds, the fluorometer will not be of value.

Gas chromatography-mass spectrometry (GC-MS), which separates the compound of interest from other potentially interfering compounds and the mass spectrometer, analyzes the purified sample over a time interval.

High-pressure liquid chromatography (HPLC) can be used as a purification method and quantitative technique. Injecting the sample onto the column separates compounds. The various components in the sample pass through the column at different rates due to partitioning differences between the mobile liquid and stationary phases. HPLC also provides greater chromatographic selectivity than gas chromatography. Fluorescent detection is frequently used for quantifying several lipid and water-soluble vitamins.

Ion exchange chromatography is commonly used in the purification of biological materials. Charged molecules in the liquid phase pass through the column until a binding site in the stationary phase appears. The molecule will not elute from the column until a solution of varying pH or ionic strength is passed through it. Separation by this method is highly selective.

Radioimmunoassay utilizes a labeled isotope to quantify a given compound. The radio-labeled isotope binds to the compound. The sample is analyzed in a scintillation counter. The greater the radioactive count, the greater amount of bound isotope.

10.5 FUTURE RESEARCH

Within the realm of vitamin assessments there are a few factors that hinder advancing our understanding of the role vitamins play in exercise and sport. A general consensus is that vitamin status does not affect exercise or sport performance in most athletes. That does not mean they are not important, but simply that most athletes consume enough vitamins to minimize the chance of a vitamin deficiency from affecting their ability to perform at a desired level. That said, there is little evidence illustrating a dose response for each vitamin on athletic performance to better determine whether athletes truly need more vitamins than less-active individuals.

So what does that issue have to do with vitamin assessments? A primary issue to address that prior point is having assessment procedures that are accurate and financially feasible. Secondary issues are how quickly the samples can be analyzed, how invasive the procedure is (for example, tissue, blood, or saliva samples), and the number of compounds to assess to determine vitamin status (such as β -carotene, retinol, or retinal concentrations for vitamin A status). Currently, most of the preferred or more valid methods to assess vitamin status require costly equipment and blood samples at the minimum. There are some less-invasive methods, but even those can present other barriers (time needed to conduct the test, for example).

Thus there is a great need for studies that are designed to increase the “ease” (cost, speed, and invasiveness) of assessing vitamin status in athletes. Given the changes that occur throughout a competitive season and the off-season for many athletes, it would be valuable to know to what degree most athletes truly need to concern themselves with vitamin intake beyond the concerns needed to maintain general health.

10.6 CONCLUSIONS

The intent of this chapter is to provide sport scientists information regarding the assessment techniques to biochemically determine vitamin status. The assessment of vitamin status tends to require more analytical steps and stability-maintaining procedures than assessments of other nutrients (glucose, non-esterified fatty acids, etc.). If greater detail is desired, there are several texts that fully explain and discuss the chemical properties and analytical methods to assess each vitamin and/or vitamin.^{1,11,33} Given the complex interactions between micronutrients, such as vitamins and other ingested compounds, it will be beneficial for scientists to assess vitamin status in athletes to justify or better explain biochemical occurrences affecting training, competition, or recovery of athletes.

When initiating the process of assessing vitamin status, it is imperative that the coach, athlete, or scientist refer to texts or articles that not only explain the methods but also discuss the confounding analytical factors that lead to the conversion of inactive isomers or instability of the vitamins being measured. These factors, in conjunction with the fact that few studies have demonstrated vitamin malnutrition in athletes, seem to minimize the interest in vitamin research in exercise science. However, the assessment of vitamin status may be prove beneficial in better understanding performance outcomes of athletes with eating disorders, those who ingest megadoses of vitamins and other supplements, and those performing high training volumes. Therefore future research in vitamin assessment should investigate how nutrient and training status (for example, malnutrition and high training volume) alter the circulating and stored levels of vitamins and the subsequent effect on performance.

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11 Assessment of Mineral Status of Athletes

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11.1 INTRODUCTION

The burgeoning awareness of the fundamental biological roles that trace elements and minerals play in the development of physical fitness and the attainment of peak performance (Table 11.1) fuels interest in measures of assessment of mineral element nutritional status of physically active people.^{1,2} Selection of an appropriate assessment tool is complicated by theoretical and practical limitations. The ideal marker should be specific and sensitive, and should distinguish adequate from deficient nutritional status. Among vigorous individuals, however, subclinical or marginal nutritional, compared to overt or clinical, deficiency is more likely to occur and thus requires one or more biomarkers for characterization. Biochemical indicators of subclinical deficiency should be practical, convenient, and cost effective, and reflect cellular mineral content and the function of specific cells. Because no single method achieves all of these criteria, a compromise is needed to achieve a valid and realistic indicator to routinely identify subclinical mineral nutritional status in humans.³ This chapter details some useful approaches to assess human mineral nutritional status with an emphasis on biochemical methods and indicators. The focus is on mineral elements that are either acknowledged to have key roles in promoting physical performance or are used as performance-enhancing supplements; they include calcium, chromium, copper, iron, magnesium, phosphorus, and zinc. The chapter describes traditional and new biochemical measures of mineral element nutriture, presents values in

TABLE 11.1
Biological Roles of Mineral Elements in Support of Physical Performance

Element	Biochemical Role	Physiological Function
Calcium	Second messenger	Muscle contraction
	Organic matrix of skeleton	Bone health
Chromium	Insulin action	Insulin sensitivity
		Glucose and lipid metabolism
Copper	Oxidative metabolism	Aerobic energy production
	Free radical breakdown	Antioxidant protection
Iron	Oxygen utilization	Aerobic energy production
Magnesium	Energy transformation	Intermediary metabolism
		Maintenance of ATP and CP
		Muscle contraction
Phosphorus	Energy storage	Formation of ATP and CP
	Oxygen release to tissues	Aerobic energy metabolism
Zinc	Macronutrient metabolism	Muscle function
	Gas transport	Oxygen release and CO ₂ removal
	Free radical neutralization	Antioxidant protection

diverse groups of physically active persons, and identifies alterations in physiological functions and performance linked with altered mineral nutritional status.

11.1.1 DIETARY INTAKE

The estimated daily intake of nutrients is a common indicator of nutritional status. Self-reports or recall of food and fluids consumed, in conjunction with computerized programs and mineral nutrient data bases, permit calculation of individual nutrient intakes from commonly consumed food and beverages. Evaluation of adequacy of intake utilizes a comparison of self-reported intake with established national recommendations such as the Dietary Reference Intakes (DRI).^{4,5} This approach relies on accurate recording or recall of amounts of food and fluids consumed, reporting of intake on multiple days of the week that are representative of usual consumption, and nutrient databases that accurately reflect nutrient contents of the food and beverages consumed. This approach can be difficult with physically active adults unless specific precautions are employed.⁶ A limitation to the use of DRI is that physical activity is considered only in calculating recommendations for calcium, iron, and magnesium intakes.⁷ The tendency of individuals to report less food than they actually consume limits the accuracy of this approach.^{8,9}

11.1.2 BIOCHEMICAL MEASURES

Use of blood biochemical analyses to assess nutritional status provides an objective alternative to self-reported food intakes. The concentrations of minerals in serum or plasma, mineral concentrations in the cellular components of blood, and

the activities of mineral-containing enzymes (metalloenzymes) in blood or cells are routine assessment measures. The rationale for these measurements is the assumption that these variables are proportional to their intracellular contents and reflect tissue or organ content and function. Some factors moderate the validity of these measures to index nutritional status. Mineral element concentrations may be affected by factors unrelated to whole-body mineral status (hormones, stress, inflammation, injury, etc.). Also, changes in the extracellular fluid volume affect concentration values.^{10,11}

11.1.2.1 Sample Collection

Accurate determination of mineral elements in biological specimens requires special precautions and attention to potential analytical concerns.^{12,13} Analysis of seemingly homogenous specimens such as blood may be markedly affected by sampling and processing procedures. Hemolysis or microhemolysis of a whole-blood sample can yield erroneously high plasma or serum concentrations of iron or zinc because erythrocyte concentrations of these and other minerals are more than ten times greater than those in plasma.¹⁴ Also, concentrations of zinc are 5 to 15% greater in serum than plasma due to the release of zinc from platelets during clotting. Selection of an anticoagulant also is important because of the potential expansion of plasma volume associated with intracellular fluid shifts that occurs during exercise and the possible contamination of heparin with zinc.¹³

11.1.2.2 Sample Contamination

The critical problem affecting the validity of mineral element analyses is sample contamination from external sources. Sources of contamination in the laboratory include dust, rubber, paper products, wood, metal surfaces, skin, dandruff, and hair. In experimental and laboratory environments, mineral elements exist in nanogram (ng) and milligram (mg) amounts. Thus, a significant portion of an analytical value may be the result of contamination unless appropriate precautions are followed. Contamination, therefore, contributes to the wide variation of reported reference values, specifically in the mineral elements reported in very low concentration ranges (for example, part per billion ranges or ng/g).

Plastic and borosilicate glass are best suited for trace element analysis. Specifically, fluorocarbon, polyethylene, and polypropylene plastics are recommended. Surfaces in contact with samples for analysis should be cleaned of adherent mineral elements by soaking with dilute, analytical-grade nitric acid or commercial, metal-binding solutions. Water should meet or exceed American Chemical Society standards of 14 MΩ/cm² for elemental contamination or resistivity.¹³ Reagents and anticoagulants should be free of mineral elements. Disposable syringes and stainless steel needles should be used for blood collection. Stainless steel contains high chromium and nickel contents; therefore stainless steel needles are not acceptable for phlebotomy when measuring chromium, nickel, and other ultra-trace elements unless they are siliconized. Commercially available, evacuated, blood collection tubes may be problematic if specimens come in contact with the stopper. Mineral elements may leach from the stopper into the blood, thus contaminating the specimen. Use of commer-

cial, trace-element-free, evacuated tubes should be examined for mineral element contamination before use.

11.1.2.3 Analytical Methods

Measurement of mineral elements in biological specimens requires analytical sensitivity, specificity, precision, accuracy, and expedience. Analytical sensitivity is paramount because concentrations of trace and ultra-trace elements occur in the mg to ng/g range.

Although a variety of analytical techniques are available, atomic absorption spectroscopy (AAS) and emission spectroscopy, including inductively coupled plasma emission spectroscopy (ICPES) and inductively coupled plasma mass spectroscopy (ICPMS), are the most commonly used in clinical settings. The AAS method is the routine assessment tool of single mineral element analysis. Samples may be diluted and aspirated directly into the flame. Electrothermal or flameless AAS methods are available for microsample volumes and very low concentrations (< 50 ng/g). Background correction using Zeeman or deuterium arc techniques is often necessary with electrothermal AAS to overcome matrix or background interferences.¹⁵ The ICPES is a multielemental method that is replacing AAS for many mineral element applications. It enables simultaneous multielemental measurements in small sample volumes and over a wide analytical range.

11.1.2.4 Quality Control

Effective quality assurance procedures must be included in mineral element analysis plans because methods for mineral analysis are susceptible to interferences with biological matrices and external contamination. In each batch of samples, there should be reagent blanks, replicate analyses to estimate precision, and reference materials with known or certified concentrations of mineral elements prepared similarly to the unknown samples to enable assessment of accuracy and batch-to-batch precision.¹⁵ Importantly, the reference material should possess the same matrix and approximately the same amounts of analytes as the unknown samples. A variety of reference or control materials are available commercially.

11.2 CALCIUM

Calcium is a major mineral in the body, serving to provide body structure with more than 99% of body calcium stored in bone. The remainder of body calcium exists in tissues and extracellular fluid, and acts to regulate a wide variety of body functions including muscle contraction, blood coagulation, enzyme activation, nerve transmission, signal transduction in hormone actions, and membrane transport.

Calcium in the serum exists in three different physiological forms. Approximately 47% of calcium is free or ionized, making it available for incorporation into intracellular compartments. Another 47% is bound to proteins, mainly albumin; this binding is highly dependent on pH. About 20% of the protein-bound calcium is bound to globulins. The remaining 6% of calcium is associated with diffusible anions, including bicarbonate, lactate, citrate, and phosphate. Factors such as diet, stress, or illness

alter the distribution of calcium among these three metabolic pools and thus affects the amounts of total and ionized calcium in the circulation.

11.2.1 METHODS FOR ASSESSING CALCIUM STATUS

The skeleton is the major reserve of calcium in the body. Thus calcium is mobilized from bone to ensure cellular functions and maintain extracellular fluid concentrations when dietary intake is inadequate. Assessment of calcium status is problematic because serum calcium levels are tightly controlled by hormones and remain constant under most conditions.¹⁶ Serum ionized calcium (> 50% of total serum calcium), also termed free calcium, is generally considered to be the physiological form of calcium because it is biologically active and is highly controlled by calcium-regulating hormones. Because approximately 47% of serum calcium is bound to proteins, total calcium concentrations are markedly affected by alterations in blood protein concentrations, principally albumin.

11.2.1.1 Total Serum Calcium

Three methods currently used to determine total calcium in biological fluids include photometric analysis, titration of a fluorescent calcium complex with ethylenediaminetetraacetic acid (EDTA) or ethylene glycol tetraacetic acid (EGTA), or AAS. The approved reference method for measuring serum concentrations of calcium is AAS¹⁷; it provides improved accuracy and precision compared to the spectrophotometric methods. Detailed procedures for the determination of calcium in serum are available.¹⁸

11.2.1.2 Serum Ionized Calcium

Serum ionized calcium, which represents the majority of calcium in the circulation, is the physiologically active form of calcium. In contrast to total serum calcium that may be normal in conditions characterized by neuromuscular irritability, such as vitamin D deficient rickets and hypoparathyroidism, serum ionized calcium concentration is reduced.

Physiological and measurement conditions, including changes in the specimen pH, the use of EDTA or heparin, and high concentrations of magnesium and sodium, affect serum ionized calcium concentrations.¹⁹ Because most anticoagulants bind calcium, serum is the preferred specimen for measuring ionized or free calcium. Common biologically active anions, including citrate, phosphate, oxalate, and sulfate, form complexes with free calcium and thus may reduce its apparent concentration. The binding of free calcium by protein and relatively small anions is affected by pH both *in vivo* and *in vitro*. Biological specimens should be analyzed at the pH of the blood because of the inverse relationship between pH and ionized or free calcium. Anaerobic conditions should be maintained because specimens lose carbon dioxide and become more alkaline when exposed to air. Also, specimens should be handled with care to minimize metabolism of erythrocyte and leukocytes, which produce acids and decrease pH.

11.2.1.3 Urinary Calcium Excretion

Under certain conditions, urinary calcium measurements, which are correlated with calcium intake,⁴ are used to assess changes in calcium metabolism. Urinary calcium

collected over a 24-hour period may be affected by antecedent diet, metabolic disorders, and or dehydration. Diets high in calcium and/or vitamin D, dehydration, hyperthyroidism, hyperparathyroidism, Paget's disease, osteoporosis, sarcoidosis, or kidney disease may result in high levels (250–300 mg/24 hour sample) of calcium in the urine. Low urine calcium levels (< 150 mg/24-hour sample) may indicate problems with the parathyroid glands (hypoparathyroidism), low amounts of calcium or vitamin D in the diet, poor absorption of calcium or vitamin D by the intestines, or kidney disease.

11.2.1.4 Calcium Reference Intervals and Data from Athletes

In healthy adults, serum calcium concentrations range from 8.6 to 10.2 mg/dL, or 2.15 to 2.55 mmol/L.¹⁸ Concentrations decrease with age in men; females have slightly lower concentrations than males. Serum ionized calcium concentrations range from 4.64 to 5.28 mg/dL (1.16 to 1.32 mmol/L) in healthy adults. Calcium in urine ranges from 150 to 300 mg/24-hour sample. Because circulating calcium levels are maintained homeostatically with calcium fluxes from bone, there is no good indicator of calcium status for adults (see [Table 11.1](#)).

Supplemental calcium in conjunction with a program of weight-bearing activity has been used to prevent and to treat bone loss in adolescent girls²⁰ and nonosteoporotic women.²¹ However, when both treatments have been applied, no additive effects were found in elderly women.²² Supplementation of healthy boys with 1000 mg calcium/d resulted in greater bone mineral content (BMC) of the whole body, and this BMC response was greater in subjects with high physical activity.²³ Supplementation of female recruits in the U.S. Navy with 2000 mg calcium and 800 IU of vitamin D/d for 8 weeks significantly reduced stress fractures by 21%.²⁴ These findings contrast with those of a supplementation study involving male military recruits receiving 500 mg of calcium/d in which the supplement, compared to a placebo, had no effect on the frequency of overuse injuries during 9 weeks of physical training.²⁵ Because total daily calcium intake was at least 800 mg/d, the authors concluded that this amount of calcium was adequate to protect against overuse bone injuries in men. Urinary calcium excretion decreased ~15% in healthy, moderately active men participating in a 3-week program of daily high-impact and resistance training activities.¹² The authors suggested that the reduction in urinary loss of calcium might be at least partially responsible for improved bone mineralization that has been observed during periods of greater physical activity.

Although the importance of measuring blood biochemical indicators of calcium status is well established in the evaluation of endocrine control of bone metabolism, measurements of calcium status in physically active persons are not common ([Table 11.1](#)). Crespo et al.²⁶ measured biological markers of nutrition in 18 marathon runners and 22 sedentary controls and reported no differences in serum calcium levels between the groups. Assessment of bone status, which is a long-term indicator of the adequacy of calcium status, relies on determinations of bone mass and quality with dual x-ray absorptiometry and determinations of circulating hormones involved in maintenance of bone accretion and turnover ([Table 11.2](#)).

TABLE 11.2**Indicators of Subclinical Deficiency of Selected Minerals and Functional Impairments**

Element	Indicator	Values Linked with Impaired Function	Impaired Function
Calcium	None	Not established	Low bone mineral density
Chromium	Serum chromium?	No reliable indicator	Impaired glucose/insulin metabolism?
Copper	Superoxide dismutase activity: serum and RBC ^a	Not established	Increased oxidative damage
Iron	Soluble transferrin receptors	> 8.5 mg/L	Decreased endurance, Reduced energy efficiency
Magnesium	RBC magnesium	< 6.0 mmol/g Hb ^b	Reduced energy efficiency, Reduced cardiorespiratory function
Phosphorus	Serum phosphate?	Not established	Decreased cell energy intermediates?
Zinc	Serum or plasma zinc	< 11.0 μ mol/L	Reduced cardiorespiratory function, Decreased endurance and strength

^a RBC = red blood cells or erythrocytes; ^b Hb = hemoglobin.

11.3 CHROMIUM

Chromium is an ultra-trace element; it facilitates the biological action of insulin in carbohydrate, protein, and lipid metabolism.²⁷ Chromium, which binds to an intracellular, low-molecular-weight chromium-binding protein, potentiates the auto-amplification of insulin signaling by stimulating the insulin receptor kinase activity in insulin-sensitive cells.²⁸ Insulin resistance may be a consequence of chromium deficiency because insulin apparently is inefficient as a regulator of glucose uptake and utilization without chromium. Because of analytical problems with measurements of very small concentrations of chromium in foods, beverages, and biological samples as well as interferences with chromium contamination, human metabolic studies of chromium are very limited. Thus, inference of human chromium deficiency relies on improvement in glucose tolerance after supplementation.²⁷

11.3.1 METHODS FOR ASSESSMENT OF CHROMIUM STATUS

Analytical limitations because of trace concentrations of chromium in human tissues and fluids have limited the identification of a reliable measure of body chromium status.²⁷ Early reports of serum and urinary chromium concentration were erroneous because of contaminations and other analytical problems. With improved method sensitivity and capability of identification and elimination of external chromium contamination, substantially decreased estimates of chromium concentration in biological and food samples appeared.

The preferred method for determination of chromium in biological samples is AAS with a graphite furnace and Zeeman correction.²⁹ Caution is advised to avoid any contact of a sample with any metal surface, including the use of stainless steel needles unless they are siliconized.

11.3.1.1 Chromium Reference Intervals and Data from Athletes

The current adult reference range for serum chromium concentration is < 0.05 to 0.52 µg/L or 1 to 10 nmol/L. Urinary chromium excretion is referenced at 100 to 200 ng/24 h. Consumption of chromium supplements will increase daily urinary chromium output depending on the dose and duration of the supplement usage.

Data describing serum chromium concentrations and urinary chromium excretion of athletes are limited.³⁰ Anderson and co-workers³¹ reported significantly increased serum chromium concentrations in men consuming self-selected diets and after running 10 km. Basal values were 2.3 ± 1.2 nmol/L, increased to 3.3 ± 1.7 nmol/L immediately after running, then to 3.6 ± 1.7 nmol/L 2 hr after exercise. Urinary chromium output also increased significantly from nonexercise values of 200 ± 120 to 370 ± 240 ng/d on the day of running. Among young men consuming self-selected diets and participating in an 8-week resistance training program, serum chromium concentrations increased from 13 ± 4 to 14.5 ± 4 nmol/L.³² With chromium supplementation (~180 mcg chromium daily as chromium picolinate), serum chromium increased from 13 ± 4 to 16 ± 3 nmol/L. Concomitantly, urinary chromium excretion increased only with chromium supplementation. Similarly, serum and urinary chromium increased significantly in healthy women fed a diet containing 29 ± 2 µg chromium daily for 12 weeks and supplemented with ~190 mcg chromium as chromium picolinate (45 µg/L and ~300 nmol/24 hr) compared to placebo or picolinic acid (1720 µg) [~ 27 µg/L and ~40 nmol/24 hr].³³ Difficulties in collecting and analyzing samples, and the direct influences of exercise and chromium intakes affecting serum and urinary chromium, contribute to the lack of an acceptable indicator of chromium status in humans (Table 11.2).

11.4 COPPER

The biochemical role for copper is primarily catalytic with many copper metalloenzymes acting as oxidases to reduce molecular oxygen. In these oxidation-reduction reactions, copper serves as the reactive center in the copper metalloenzymes. Some copper metalloenzymes include ceruloplasmin, superoxide dismutase, dopamine-β-hydroxylase, lysyl oxidase, cytochrome c oxidase, and tyrosinase.³⁴ Thus, copper plays a key role in supporting increased energy expenditure, antioxidant protection, and synthesis of important protein needed to sustain physical activity.

Copper deficiency decreases the activity of copper metalloenzymes and results in marked biological impairments.³⁴ Defective connective tissue cross-links in heart, muscle, and bone may be attributed to decreased lysyl oxidase activity. Hypopigmentation has been associated with depressed tyrosinase activity because copper is required for melanin synthesis. Oxidative damage in various organs, tissues, and organelles has been shown to be the result of decreased superoxide dismutase activity. Low copper intake in humans has been associated with exaggerated blood

pressure responses during isometric exercise and attributed to altered dopamine- β -hydroxylase activity in vascular tissue.³⁵

11.4.1 METHODS FOR ASSESSMENT OF COPPER STATUS

Biochemical indicators of copper nutritional status continue to undergo evaluation and validation.³⁶ Despite the lack of an unequivocal marker of human copper nutritional status, a number of indices can be useful in the diagnosis of subclinical copper deficiency (Table 11.2).

11.4.1.1 Serum and Plasma Copper

A common measure of copper status is serum or plasma copper concentration, with low copper concentration in plasma or serum indicative of depleted body copper stores. However, plasma copper concentrations are not reliable indicators of short-term marginal copper status in humans. Homeostatic mechanisms regulate plasma copper concentrations within a narrow range. Thus, plasma copper concentrations decrease only after significant depletion of body copper stores.³⁷ Factors independent of copper intake affect circulating copper concentrations. Women generally have higher plasma or serum copper concentrations than men; estrogen increases plasma copper concentrations in women taking oral contraceptive agents and postmenopausal women receiving estrogen therapy.³⁸ Plasma copper concentrations are increased in pregnancy, inflammation, infection, and rheumatoid arthritis.³⁹ In contrast, general stress and glucocorticoid hormones decrease plasma copper concentrations.³⁹ Thus conditions that elevate serum copper may belie decreased serum copper even during copper deprivation. Also, circumstances that reduce serum copper should be eliminated before a valid assessment of copper nutritional status may be undertaken.

The method of choice for determination of plasma or serum copper is AAS after dilution of the specimen with deionized water. Hemolysis is not a major concern for copper determinations because concentrations of copper in erythrocytes and plasma are similar.

11.4.1.2 Ceruloplasmin

More than 80% of the copper in plasma is associated with the protein ceruloplasmin; changes in plasma copper are reflected in changes in the amount of this protein in the circulation. Both the enzymatic activity of ceruloplasmin and the immunoreactive protein ceruloplasmin respond similarly to age, sex, and hormone use; they increase in pregnancy and in response to inflammation. Enzymatic activity of ceruloplasmin has been shown to be an indicator of copper status in animals and humans deprived of copper.³⁷

Serum ceruloplasmin may be measured immunochemically or by its oxidase activity. The copper-depleted, apo-ceruloplasmin is likely present in normal and copper-deficient serum.³⁷ Thus, chemical assays of its oxidase activity are preferred as an index of copper status. The specific activity of ceruloplasmin, defined as the ratio of enzymatic activity to the immunoreactive protein, may be a sensitive marker of copper status because it was inversely related to blood pressure response to hand-

grip work.³⁵ This ratio, which is not affected by age, sex, or hormone use,³⁷ should be used to assess copper status of athletes.

11.4.1.3 Copper, Zinc Superoxide Dismutases

Superoxide dismutases (SOD) belong to a family of antioxidant enzymes that catalyze the dismutation of the superoxide radical to yield hydrogen peroxide and oxygen. There are two distinct forms of SOD in mammals and each utilizes copper in its reactive center. These isozymes are characterized by their cellular location and distribution among various tissues. The erythrocyte isoform (SOD1) is localized in the nucleus and cytoplasm and found principally in erythrocytes and liver cells.⁴⁰ However, the extracellular isoform (SOD3) is concentrated in the extracellular matrix of tissues, specifically lung and kidney, and is the dominant extracellular antioxidant enzyme found in the serum.⁴¹

11.4.1.3.1 Erythrocyte Superoxide Dismutase

Erythrocyte superoxide dismutase activity decreases during copper deficiency in humans and some animal species. It also is sensitive to changes in copper status as shown in several studies of experimental copper deprivation.³⁷ Compared to other biochemical markers of copper status such as plasma copper and ceruloplasmin, erythrocyte superoxide dismutase activity is independent of age, sex, and hormone use.⁴²

11.4.1.3.2 Extracellular Superoxide Dismutase

The extracellular superoxide dismutase is a secretory protein present in relatively reduced amounts in the circulation relative to its tissue source.⁴¹ It is responsive to changes in copper⁴³ and zinc^{44,45} intake in animal models. Some controversy exists regarding the specificity of the activity of extracellular superoxide dismutase as a functional indicator of copper or zinc status, as both conditions have been shown to reduce its activity.^{46,47}

Biochemical assays for superoxide dismutase are based on the indirect measurement of activity that consists of a superoxide generating system and a superoxide indicator that is measured spectrophotometrically.⁴⁸ Addition of copper (zinc superoxide dismutase, specifically SOD1) inhibits the absorption change. The use of the autooxidation of pyrogallol has been the principal method for determination of erythrocyte superoxide dismutase activity.⁴⁹ In contrast, the autooxidation of xanthine by xanthine oxidase is the recommended method for determination of extracellular superoxide dismutase.⁵⁰ Recent findings reveal that the determination of SOD3 activity in serum with xanthine and xanthine oxidase at pH 10 is a very sensitive indicator of copper status.⁵¹

A practical problem arises from the use of superoxide dismutase activities to assess copper status; there are no uniform reference ranges available. To facilitate the use of superoxide dismutase, it is suggested that reference ranges be developed in each laboratory and the conditions used for analysis be maintained.

11.4.1.4 Cytochrome c Oxidase

Decreased tissue cytochrome c oxidase activity is an early and consistent trait of copper deficiency in animals. Reductions of 50% of normal cytochrome c oxidase

activity are associated with impaired neurological, cardiac, and muscle functions.⁵² Studies in humans report that cytochrome c oxidase activities in platelets are decreased when dietary copper is restricted.^{37,53} Cytochrome c oxidase activity in platelets and leukocytes paralleled copper status in animals⁵⁴; the cytochrome c oxidase activity correlated directly with liver copper concentration, an established index of copper status in animals.

Available methods for determination of cytochrome c oxidase activity in blood cells and tissues utilize the spectrophotometric analysis of the oxidation of ferricytochrome c. A microassay has been described that uses a coupled reaction between cytochrome c and 3-3'-diaminobenzidine tetrachloride in microwell plates.⁵⁵

Age affects cytochrome c oxidase activity.^{37,53} Platelet and leukocyte cytochrome c oxidase activity are higher in older than in young adults but are not affected by sex or hormone use. Other factors that may limit the use of this marker include considerable between-individual variability, the labile nature of this enzyme, and its sensitivity to minor variations in technique.

11.4.1.5 Copper Reference Intervals and Data from Athletes

Serum copper concentrations are higher in women of child-bearing age, 80 to 190 $\mu\text{g/dL}$ or 12.6 to 24.4 $\mu\text{mol/L}$, than in men, 70 to 140 $\mu\text{g/dL}$ or 11 to 22 $\mu\text{mol/L}$. Serum copper is highest in pregnant women, 118 to 302 $\mu\text{g/dL}$ or 18.5 to 47.4 $\mu\text{mol/L}$. The range of normal values for children 6 to 12 years of age is 80 to 90 $\mu\text{g/dL}$ or 12.6 to 29.9 $\mu\text{mol/L}$.¹⁷

There is a paucity of data describing the copper status of athletes. In a sample of 44 male university athletes, plasma copper was $90 \pm 14 \mu\text{g/dL}$ with hypocupremia ($< 70 \mu\text{g/dL}$) present in four of the men.⁵⁶ Plasma copper (95 ± 11 and $94 \pm 10 \mu\text{g/dL}$) and enzymatic ceruloplasmin (419 ± 37 and $397 \pm 38 \text{ mg/L}$) were unchanged from precompetition to end of the competitive season in 12 elite female university swimmers.⁵⁷ Similarly, plasma copper and enzymatic ceruloplasmin were within the range of normal values for male and female swimmers before and during a competitive season.⁵⁸ Interestingly, SOD1 activity increased significantly in response to swim training, despite reduced copper intake.⁵⁸ Thus, the increased activity of SOD1 was an adaptation to increased oxidative stress associated with aerobic training.

Copper status of runners also has been assessed. Anderson et al.³¹ reported serum copper of $93 \pm 15 \mu\text{g/dL}$ for 9 trained runners. In contrast, Singh et al.⁵⁹ found increased plasma copper (122.2 ± 12.5 vs. $106.6 \pm 15.6 \mu\text{g/dL}$), decreased erythrocyte copper (1.06 ± 0.02 vs. $1.26 \pm 0.03 \mu\text{g/g}$), and similar enzymatic ceruloplasmin (287 ± 49 vs. $281 \pm 50 \text{ mg/L}$) in 45 female runners compared to 27 nonrunners. Thus exercise apparently induced a redistribution of copper in the women.

The use of more than one biochemical measure of copper status will increase the probability of reliably identifying an individual as copper deficient or adequate. Thus, the use of multiple indicators, such as serum copper, platelet cytochrome c oxidase, and superoxide dismutase activities, will enhance success of a valid assessment of copper nutritional status in physically active persons (Table 11.2).

11.5 IRON

Iron serves as a component of a number of proteins hemoglobin and myoglobin for oxygen transport and intracellular oxygen storage, and enzymes required for cellular energy production. More than 60% of iron in the body is found in hemoglobin in circulating erythrocytes, 25% as a readily mobilizable iron store, and the remaining 15% in myoglobin of muscle and a variety of iron-containing enzymes (such as cytochromes).

Iron deficiency is one of the most prevalent micronutrient deficiencies in both industrialized and developing countries.⁶⁰ It is most common among children and women during their reproductive years; it may be seen among men as the result of chronic blood loss associated with parasitic load. Severe iron deficiency is manifest as anemia with adverse consequences such as impaired immune function, decreased work capacity, cold intolerance, and compromised learning ability. In contrast, iron overload, which may be caused by progressive accumulation of iron in tissues (idiopathic hemochromatosis), may contribute to ischemic heart disease and cancer. It also may be the result of excessive use of iron supplements, injections of therapeutic iron, or blood transfusions.

11.5.1 METHODS FOR ASSESSMENT OF IRON STATUS

A variety of biochemical measures is available to assess iron status because it ranges from deficiency states to iron overload in humans. Some common measurements include hemoglobin, hematocrit, various erythrocyte indices, ferritin, serum iron, total iron-binding capacity, transferrin, transferrin saturation, soluble transferrin receptors, free erythrocyte protoporphyrin, and zinc protoporphyrin. These status indicators vary in their sensitivity and specificity.

11.5.1.1 Hemoglobin and Hematocrit

Measurement of hemoglobin concentration in whole blood is the most widely used assessment tool for iron deficiency anemia. As an indicator of iron deficiency, it is relatively insensitive and exhibits low specificity. Hemoglobin concentrations decrease only during the late stages of iron deficiency after tissue iron stores have been greatly reduced. Moreover, hemoglobin concentration may be affected by other nutritional perturbations, such as folic acid, copper, and vitamin B₁₂ deficiency, and other conditions including pregnancy, tobacco smoking, infection, and inflammation, as well as dehydration.⁶¹ A common method for measurement of hemoglobin in blood includes spectrophotometry after anticoagulation with heparin or EDTA and conversion to cyanomethemoglobin.

Hematocrit or packed erythrocyte volume decreases after erythrocyte production has been reduced. Thus it also is relatively insensitive and nonspecific because hematocrit is influenced by the same factors that affect hemoglobin concentrations, mainly changes in plasma volume.

11.5.1.2 Ferritin

Serum ferritin concentration is in equilibrium with body stores, and variations in the quantity of iron in the storage compartment affect serum ferritin concentration.⁶²

Serum ferritin concentration declines very early in the development of iron deficiency, well in advance of any reductions in hemoglobin or serum iron concentration. Thus serum ferritin may serve as a useful indicator of tissue iron deficiency. However, certain chronic conditions, including chronic infections, inflammatory diseases, some malignancies, and liver damage, increase serum ferritin concentrations independently of iron intake. Among healthy women depleted of iron by diet and phlebotomy, then repleted with iron, serum ferritin was the most sensitive measure of changes in iron status and body iron stores.⁶³ Serum ferritin concentrations are currently determined by using immunological techniques. Commercial radioimmunoassay and enzyme-linked immunosorbent assay kits are available.

11.5.1.3 Serum Iron, Total Iron-Binding Capacity, Transferrin, and Transferrin Saturation

Measurement of iron and iron bound to transport proteins in blood provides another indicator of iron nutritional status. Serum iron and total iron-binding capacity reflect the transit of iron from the reticuloendothelial system to the bone marrow. Transferrin is the transport protein for iron in blood (serum) and is generally only one third saturated with iron in normal circumstances. Transferrin may be measured immunologically, but practically it is determined as total iron-binding capacity. The most useful measure of iron transport capacity is transferrin saturation, which is calculated as the ratio of serum iron to total iron-binding capacity. Importantly, serum iron and iron-binding capacity respond in reciprocal manner during iron deficiency and overload. A transferrin saturation less than 16% indicates an inadequate iron intake, whereas iron saturation exceeding 55% reflects iron overload and possibly hemochromatosis.⁶⁴

Serum or plasma iron is measured using chromogens, such as bathophenanthroline sulfonate and ferrozine, and spectrophotometry. The use of AAS to measure serum or plasma iron is not recommended because AAS will determine the heme iron released during hemolysis; the colorimetric procedure does not detect heme iron. Total iron-binding capacity is determined by initially saturating the serum with excess iron and then adding magnesium carbonate to adsorb and remove excess iron not bound to transferrin.

11.5.1.4 Soluble Transferrin Receptor

The cell membranes of the developing erythrocyte precursors in bone marrow are very rich in transferrin receptors to which the iron-transferrin complex binds before it is internalized to deliver iron in cells. Measurement of transferrin receptor concentration in blood is a useful index of subclinical iron deficiency because the number of transferrin receptors increases during iron deficiency and decreases during iron excess.⁶⁵ Studies in humans show that soluble transferrin receptor concentrations in blood discriminate tissue iron deficiency better than ferritin concentrations and are not influenced by inflammation.^{66–68} Circulating serum transferrin receptor concentrations decrease only after iron stores are replenished and in advance of other markers of iron deficiency. Also the ratio of soluble transferrin receptor to ferritin concentrations is a practical index of iron overload.⁶⁹ Soluble transferrin receptor

concentrations can be determined by using commercially available enzyme linked immunosorbent assay (ELISA); however, care is required to minimize between-batch variability.

11.5.1.5 Free Erythrocyte Protoporphyrin and Zinc Protoporphyrin

The concentrations of these proteins have been found to be sensitive indicators of iron-deficient erythrocyte production.⁷⁰ Changes in free erythrocyte protoporphyrin or zinc protoporphyrin are relatively insensitive to acute changes in iron status due to the slow turnover rate of erythrocytes, 90 to 120 days. Free erythrocyte protoporphyrin and zinc protoporphyrin concentrations are measured spectrophotometrically.

11.5.1.6 Iron Reference Intervals and Data from Athletes

Serum iron concentrations range from 65 to 165 $\mu\text{g/dL}$ or 11.6 to 31.3 $\mu\text{mol/L}$ in men and 50 to 170 $\mu\text{g/dL}$ or 9.0 to 30.4 $\mu\text{mol/L}$ in women.¹⁷ Total serum iron-binding capacity in healthy adults ranges from 250 to 425 $\mu\text{g/dL}$ or 44.8 to 76.1 $\mu\text{mol/L}$. Serum ferritin concentrations range from 20 to 250 $\mu\text{g/L}$ in men and 10 to 120 $\mu\text{g/L}$ in women. Ferritin concentrations less than 10 $\mu\text{g/L}$ indicate depleted iron stores, whereas concentrations greater than 300 mcg/L suggest iron overload.

Iron status in athletes and physically active persons has been summarized in several reviews.^{71,72} Iron deficiency anemia, defined as hemoglobin concentrations less than 110 and 120 g/L in women and men, respectively, has been reported in physically active persons.⁷³ This severe iron deficiency is associated with reduced aerobic capacity in athletes and decreased work productivity in agricultural laborers. In some studies, the anemia is present in all of the subjects.⁷⁴ Generally anemia is present in a small percentage (~2%) of athletes examined.^{75–78} More common (25–50%) is subclinical iron deficiency, characterized by decreased iron stores and increased iron-binding capacity without anemia. Since the initial finding that depleted body iron stores adversely affect work metabolism,⁷⁹ there has been increased awareness that subclinical iron deficiency is associated with impaired endurance and muscle function. Studies of adolescent girls with decreased serum ferritin and normal hemoglobin concentrations experienced decreases in training and impaired endurance.^{80,81} Medical treatment with iron supplementation (100 mg/d) improved training duration and endurance performance.

Despite these positive findings, experimental data do not reveal a consensus on the effects of iron supplementation on performance of adults with subclinical iron deficiency defined as low ferritin ($< 12 \mu\text{g/L}$) or increased soluble transferrin receptor concentration ($> 8.5 \text{ mg/L}$). Women supplemented with iron (100 mg/d for 8 weeks) significantly increased peak oxygen uptake and endurance and decreased blood lactate with significant increases in ferritin (22.5 vs. 14.3 $\mu\text{g/L}$) and hemoglobin (141 vs. 128 g/L) compared to placebo-treated subjects.⁸² Other supplementation trials of iron-deficient adults, however, only report significant increases in ferritin or soluble transferrin receptor concentrations without improvements in peak work capacity but significant reductions in lactate or increased endurance.^{79,83} One key factor that could explain these divergent findings is the confounding effect of inflammation that sequesters iron and reduces circulating ferritin levels.^{66,67}

Accumulating evidence reveals the beneficial effects of iron supplementation on performance of adults with subclinical iron deficiency. Women with subclinical iron deficiency supplemented with iron (8 mg/d for 6 wk) and trained on cycle ergometers significantly increased serum ferritin (10.4 ± 0.82 to 14.52 ± 1.5 $\mu\text{g/L}$) and decreased soluble transferrin receptor (7.92 ± 0.87 to 6.78 ± 0.42 mg/L) concentrations without a change in hemoglobin compared to placebo-treated controls (8.07 ± 0.77 to 8.11 ± 0.90 $\mu\text{g/L}$ and 7.94 ± 0.73 to 7.93 ± 0.77 mg/L, respectively).⁸⁴ Although both iron-supplemented and placebo-treated women reduced 15-km time trial times, the improved endurance was significant only in the women supplemented with iron. A follow-up study showed that the iron-supplemented women with initial subclinical iron deficiency completed a simulated 15 km time trial is significantly less time, consistent with the previous observation, and exercised a significantly higher work rate with a lower percent of aerobic capacity than the placebo-treated women.⁸⁵ Similarly, iron supplementation (10 mg/d for 8 weeks) of female soldiers engaged in military training significantly attenuated the decline in iron status (that is, decreased ferritin and increased soluble transferrin receptor concentrations) and was associated with improved endurance and mood compared to placebo-treated female controls.⁸⁶ Among women with subclinical iron deficiency, iron supplementation (10 mg/d for 6 weeks) maintained soluble transferrin receptor concentrations and significantly increased progressive fatigue resistance during dynamic knee extensor exercise (such as muscle force at voluntary fatigue) to exhaustion compared to unsupplemented women whose soluble transferrin receptor concentrations increased significantly.⁸⁷ In these studies, soluble transferrin receptor concentrations in serum were a discriminating indicator of subclinical iron deficiency and physical performance (Table 11.2).

11.6 MAGNESIUM

Magnesium is an intracellular cation; it is required in a wide variety of fundamental cellular processes that support diverse physiological functions.^{4,88} Magnesium is involved in more than 300 enzymatic reactions in which food is metabolized and new products are formed. Some of these reactions are involved in glycolysis, fat and protein metabolism, hydrolysis of adenosine triphosphate (ATP), and second messenger system and signal transduction. Magnesium also regulates membrane stability and neuromuscular, cardiovascular, immune, and hormonal functions. Thus magnesium may be a limiting factor in physical performance.⁸⁹

11.6.1 METHODS FOR ASSESSMENT OF MAGNESIUM STATUS

Evaluation of human magnesium status is challenging; there is no simple, rapid, and accurate laboratory test to indicate subclinical magnesium status.⁸⁸ A number of approaches have been used to assess magnesium status, including determination of circulating magnesium, measurement of cellular magnesium content, and indirect assessment of body magnesium stores.

11.6.1.1 Serum and Plasma Magnesium

Serum magnesium is the most commonly used indicator of magnesium status. Although a practical tool, serum or plasma magnesium is only an index of the presence or absence of severe magnesium deficiency. Low magnesium concentration or hypomagnesemia reliably indicates magnesium deficiency; however, its absence does not exclude significant magnesium depletion. The concentration of magnesium in serum has not been shown to be correlated with the concentration of magnesium in any other tissue pools except interstitial fluid, a component of the extracellular fluid.⁹⁰

Serum, rather than plasma, is preferred because anticoagulants may be contaminated with magnesium or affect the assay procedure. It is critical to avoid hemolysis because the magnesium concentration of erythrocytes is three times as great as serum. Magnesium concentration in serum is determined directly by flame AAS after diluting fifty-fold with a lanthanum chloride or oxide diluent.

11.6.1.2 Ionized Magnesium

Magnesium in serum exists in several forms at physiological pH: protein-bound (19–34% of total), free magnesium ion (61–67% of the total), and complexed to certain anions (5–14% of the total).⁹¹ The free or ionized magnesium is considered to be the physiologically active form, but it also is not adequate to detect subclinical magnesium deficiency.⁹² Ionized magnesium is measured in serum with ion-specific electrodes. Magnetic resonance spectroscopy provides the unique opportunity to measure intracellular magnesium in tissues *in vivo* at rest and during exercise⁹³; however, this novel technology is restricted to a few research laboratories.

11.6.1.3 Muscle Magnesium

More than 26% of magnesium is localized in muscle. Knowledge of the fundamental biological roles of magnesium in metabolism suggests that muscle is an appropriate tissue to sample in healthy and ill persons to assess magnesium nutriture. Percutaneous skeletal muscle biopsy has been used to assess magnesium status in humans.^{93,94} However, because this is an invasive procedure and requires special skills and equipment, it has not been widely used.

11.6.1.4 Blood Cells

The mononucleated white blood cell has been proposed as a possible indicator of cellular magnesium status. Indeed, several studies reported a significant correlation between the magnesium concentration of the mononucleated cells and skeletal muscle magnesium but not serum magnesium.^{92,93}

Erythrocyte magnesium concentration has been associated with hypertension, chronic fatigue syndrome, and premenstrual syndrome,⁹² but its value as an indicator of cellular magnesium content has only recently been examined. Among women fed controlled diets containing adequate, low, and supplemental magnesium (322, ~140, and 360 mg/d), erythrocyte magnesium decreased significantly (6.8 to 5.8 $\mu\text{mol/g}$ hemoglobin) and then increased (6.6 $\mu\text{mol/g}$ hemoglobin), in contrast to serum magnesium that only decreased from 0.85 to 0.81 mmol/L with restricted magnesium

intake.⁹⁴ Importantly, the changes in erythrocyte magnesium paralleled the significant reductions in skeletal muscle magnesium with low magnesium intakes and the significant increases in response to supplemental magnesium (52, 48, and 54 mmol/kg dry weight, respectively). Thus erythrocyte magnesium concentrations are a practical alternative for assessing muscle magnesium.

11.6.1.5 Magnesium Load Test

Oral and intravenous magnesium loading tests have been described and perhaps are more widely used as a diagnostic tool of body depletion of magnesium than measures of intracellular magnesium.⁸⁸ Persons with adequate magnesium in body pools generally excrete the vast majority (75 to 100%) of the administered dose within 24 to 48 hours, compared to persons with depleted magnesium pools who retain a significant proportion of the dose. Sensitivity of the response as well as the need for normal kidney function and the lack of any disturbances in myocardial conductivity hamper the application of this test.

11.6.1.6 Magnesium Reference Intervals and Data from Athletes

Total magnesium concentrations, as determined by AAS, range from 1.6 to 2.6 mg/dL or 0.66 to 1.07 mmol/L.¹⁷ There is no apparent diurnal variation in total serum magnesium concentration.

Impetus for the measurement of magnesium status of physically active persons began with a report of hypomagnesemia (plasma magnesium < 0.60 mmol/L) associated with muscle spasms in a competitive tennis player.⁹⁵ Surveys of adult athletes participating in a variety of sports indicate values of plasma or serum magnesium in the range of normal values.^{56,96–101} Similarly, children participating in swim training had normal values for plasma magnesium concentrations.¹⁰² Intense, anaerobic training transiently decreases plasma magnesium concentration with a parallel increase in urinary magnesium excretion.^{103,104} Routine assessment of erythrocyte magnesium concentration is recommended because it reflects decreased cellular magnesium status and its adverse impact on muscle function (Table 11.2).

11.7 PHOSPHORUS

Phosphorus in the form of inorganic or organic phosphate is the second most abundant mineral in the body. More than 85% of the phosphorus in the adult is present in the skeleton as either hydroxyapatite or as calcium phosphate. The remainder is in cells of the soft tissues (14%) and the extracellular fluid (1%); it is present as inorganic phosphate or in nucleic acids, phosphoproteins, phospholipids, and high-energy compounds including phosphocreatine (CP) and adenosine mono-, di-, and triphosphates (AMP, ADP, and ATP). Phosphorous is an essential factor in most energy-producing reactions of cells.⁴

Phosphorus depletion results in low intracellular concentrations of phosphoglycerate, ATP, and CP that impair muscle function, work capacity, and overall cardio-respiratory function. Impaired phosphorus status is associated with long-term total parenteral nutrition, metabolic perturbations resulting in ketoacidosis, and excessive use of antacids containing aluminum hydroxide or aluminum carbonate.

11.7.1 METHODS FOR ASSESSMENT OF PHOSPHORUS STATUS

Serum phosphorus, measured as phosphate, is used most frequently to assess phosphorus status (Table 11.2). Phosphate in serum exists both as the monovalent and divalent anion. The ratio of $\text{H}_2\text{PO}_4^{-1}$ to HPO_4^{-2} varies from 1:1 in acidosis to 1:4 at physiological pH and 1:9 in alkalosis. Approximately 55% of the phosphate in serum is free; 35% is complexed with sodium, calcium, and magnesium; and 10% is bound to protein.

Serum phosphorus concentrations are generally measured colorimetrically.¹⁰⁵ It is critical to separate blood cells from the serum as soon as possible because high concentrations of organic phosphate esters in cells may be hydrolyzed to inorganic phosphate during storage.

11.7.1.1 Phosphorus Reference Intervals and Data from Phosphate Supplementation Trials

Age affects the range of normal values of serum phosphorus. Values are higher in infancy and then decline throughout childhood until adulthood concentrations are reached. Serum phosphate, expressed as phosphorus, ranges from 4.0 to 7.0 mg/dL or 1.29 to 2.26 mmol/L in children, and from 2.5 to 4.5 mg/dL or 0.81 to 1.45 mmol/L in healthy adults. Serum phosphate concentrations are dependent on timing of food and fluid intake and are sensitive to changes in hormone status, particularly parathyroid hormone.

The effects of phosphate loading on exercise performance have been labeled as both inconsistent¹⁰⁶ and equivocal.¹⁰⁷ This conclusion was drawn from about a dozen studies conducted with phosphate supplementation and its effect on physical performance, and the results were clearly ambiguous. However, no study had reported decreases in performance, and five studies from independent laboratories have now shown remarkable similarities relative to increased levels of VO_2max following phosphate supplementation and improved performance on bicycle ergometer exercise tests such as a simulated 40-km cycle time trial.^{108–112} Serum phosphate has been used to evaluate the effects of phosphate supplements on compliance and physical performance.^{106,113} Phosphate loading increases both plasma and erythrocyte phosphate pools and the rise in erythrocyte 2,3-bisphosphoglycerate levels (2,3-BPG) is probably a consequence of the rise in cellular inorganic phosphate concentration $[\text{P}_i]$.¹¹⁴ As compared to placebo, ingestion of 1 g of tribasic sodium phosphate for 4 days significantly increased basal serum phosphate concentration from 0.95 ± 0.17 to 1.11 ± 0.32 mmol/L in six male endurance athletes.¹¹² However, at peak exercise, there was no difference in serum phosphate concentrations (1.50 ± 0.19 vs. 1.48 ± 0.21 mmol/L). Interestingly, most studies report greater increases in serum phosphate after a bout of acute exercise than after ingestion of phosphate-containing supplements.¹⁰⁶ Finally, Goss et al. recently reported that although phosphate supplementation did not affect physiological responses during exercise at about 70–80% of VO_2max , the rating of perceived exertion (RPE) was lower, suggesting a beneficial psychological effect.¹¹⁵

11.8 ZINC

The vital role of zinc for optimal growth and well-being of animals and plants emphasizes its biological importance. Zinc is required for the activity of more than 300 metalloenzymes participating in essentially all aspects of metabolism. These zinc-containing enzymes include RNA and DNA polymerase, carboxypeptidase, carbonic anhydrase, and alcohol dehydrogenase.¹¹⁶ Zinc also plays a regulatory role in gene expression by affecting gene structure and enzymatic activity.¹¹⁶

Zinc deficiency occurs at various stages with different signs. Severe deficiency presents with alopecia, weight loss, clinical behavioral and neurophysiological disorders, and ultimately death, if untreated. Moderate deficiency is characterized by growth retardation, mild dermatitis, impaired cognition, poor appetite, impaired immune function, and abnormal light-to-dark visual adaptation. In contrast, signs of subclinical zinc deficiency generally require a stressor for presentation and may include impaired cognitive function, altered behavior, and low resistance to infection.

11.8.1 METHODS FOR ASSESSMENT OF ZINC STATUS

Laboratory assessment of zinc status includes measurement of zinc in a body fluid and determination of the activity of a specific zinc-dependent enzyme. Although practical, the determination of the zinc concentration of plasma or serum is not a reliable indicator of subclinical zinc status of an individual.¹¹⁷ Functional assessments of the activity of zinc-containing enzymes (for example, 5-nucleotidase in plasma), metallothionein mRNA in monocytes and erythrocytes, certain zinc transporters in blood cells, and responses to controlled stressors such as exercise and ethanol administration are promising indicators of subclinical zinc status of humans.¹¹⁸ No single test has been proven to be a definitive indicator of zinc status.¹¹⁹

11.8.1.1 Plasma and Serum Zinc

Although the zinc concentration in plasma or serum often has been interpreted to indicate human zinc deficiency, it does not reflect whole-body zinc status.^{117, 118} Concurrent conditions that decrease plasma zinc concentration without causing zinc depletion including nonfasting conditions, infection, inflammation, steroid use, pregnancy, low serum albumin associated with liver disease, and malnutrition.¹¹⁷

The most practical and reliable analytical method to determine plasma or serum zinc is AAS. The recommended approach is to use a fivefold dilution of plasma or serum and standards with 5% glycerol matrix with AAS.¹²⁰ Measurement of plasma or serum zinc by inductively coupled plasma–optical emission spectroscopy (ICP-OES)¹²¹ enables improved sensitivity with smaller volumes than AAS and is restricted to a few research laboratories. Hemolysis must be avoided during sample collection and preparation to avoid aberrant zinc values because erythrocytes contain greater than ten times more zinc than plasma.

11.8.1.2 Zinc-Containing Enzymes

Several zinc-containing enzymes, such as alkaline phosphatase, carbonic anhydrase, nucleoside phosphorylase, and ribonuclease, are useful indicators of zinc adequacy.

Decreased alkaline phosphatase activity in either serum or neutrophils has been shown in a number of human zinc-deficient conditions. Zinc-deficient patients supplemented with zinc also increased alkaline phosphatase activity in response to zinc supplementation. Similarly, carbonic anhydrase and nucleoside phosphorylase activities increased in sickle anemia patients treated with zinc. Importantly, these positive responses in enzyme activity occurred in subjects with severe, not subclinical, zinc deficiency.

11.8.1.3 Zinc Reference Intervals and Data from Athletes

The accepted reference interval for zinc in plasma is 70 to 150 $\mu\text{g/dL}$ or 10.7 to 22.9 $\mu\text{mol/L}$. Serum zinc concentrations are generally 5 to 15% higher than plasma values because of osmotic shifts of fluid into the extracellular fluid when various anticoagulants are used. Because of diurnal variation and significant effects of recent food ingestion, a fasting morning blood sample is recommended for routine assessment of human zinc status.

Because of the potential of subclinical zinc deficiency to adversely affect all aspects of metabolism, there has been an effort to assess zinc status in many groups of athletes. Twenty-five percent of 76 experienced marathon runners had serum zinc concentration less than 11.5 $\mu\text{mol/L}$.¹²² In a survey of elite German athletes, 25% of the men and women were characterized as hypozincemic defined as serum zinc concentrations less than 12.0 $\mu\text{mol/L}$.¹²³ Among male participants in a 500-km road race, prerace serum concentrations were markedly depressed, with the majority of the values less than 11 $\mu\text{mol/L}$.¹²⁴ A similar pattern of low plasma zinc concentrations was found in elite female endurance runners.¹²⁵ Other studies did not identify low zinc concentrations in runners,^{31,59,98} swimmers,^{59,101} skiers,¹²⁷ and volleyball players.¹²⁸ However, some longitudinal studies of athletes did not report significant decreases in serum zinc during intensive training.^{126,128}

Low serum zinc concentrations have been associated with decreased muscle strength and diminished exercise capacity.¹²⁹ Adolescent gymnasts, screened for delayed pubertal maturation and growth, had serum zinc concentrations significantly less than age-matched nontraining adolescents (9.2 ± 0.4 vs. 12.4 ± 0.2 $\mu\text{mol/L}$).¹³⁰ Among the 21 gymnasts, the 12 girls had lower serum zinc than the 9 boys (8.5 ± 0.3 vs. 10.1 ± 0.6 $\mu\text{mol/L}$). Serum zinc concentrations were significantly correlated ($r = 0.465$) with isometric adductor strength. Similarly, a screening of 21 male soccer players revealed that 9 had low serum zinc concentrations (8.3 ± 0.2 $\mu\text{mol/L}$) and 12 had normal serum zinc (11.3 ± 0.2 $\mu\text{mol/L}$).¹³¹ The hypozincemic men had significantly decreased peak power output and a lower lactate threshold. Thus serum or plasma zinc concentration, specifically hypozincemia, may be a specific indicator of impaired physiological function associated with either inadequate (low) zinc intake and/or excessive zinc losses (Table 11.2).

11.9 INFLAMMATION AND MINERAL STATUS

Heavy and prolonged exercise can cause an acute phase response.¹³² This response is characterized by secretion of many diverse immunological proteins such as C-reactive protein, ferritin and cytokines including interleukin-6 (IL-6).¹³³ The net effect of this

inflammatory action is to sequester trace elements that have been released into the circulation because of exercise-induced breakdown of erythrocytes, muscle damage, or mobilized from storage depots in the liver. Increased production of IL-6 induces expression of hepcidin in the liver^{134,135} and the uptake of iron into macrophages. Thus, exercise-induced inflammation alters blood biochemical markers of iron status by increasing ferritin and decreasing iron concentrations in blood.¹³⁶

Exercise also affects zinc status. Metallothionein is a ubiquitous, zinc-binding intracellular protein. Its expression is up-regulated by oxidative and inflammatory stressors, such as strenuous exercise.^{137,138} Metallothionein also serves as a potent antioxidant during strenuous exercise.^{139,140} Increases in tissue metallothionein result in decreases in circulating zinc concentrations.¹³⁷

These findings demonstrate that inflammatory stressors, such as exercise, affect traditional blood biochemical measures of iron and zinc nutritional status independently of intake. Importantly, they indicate the need to standardize the timing of blood sampling to control for the moderating effects of physical training.

11.10 FUTURE RESEARCH NEEDS

There is increasing evidence that many mineral elements regulate key biological activities required to develop and maintain human physical work capacity, sustain training, and optimize performance (Figure 11.1). One factor confounding research on the interaction of mineral intake and physiological function has been the lack of appreciation of the need for standardization of collection practices (such as fasting and before physical activity). Appropriate guidelines are available to minimize contamination during phlebotomy and processing of blood samples. Development and implementation of contemporary analytical methods are needed to minimize blood volumes that will facilitate future research and regular nutritional monitoring. There

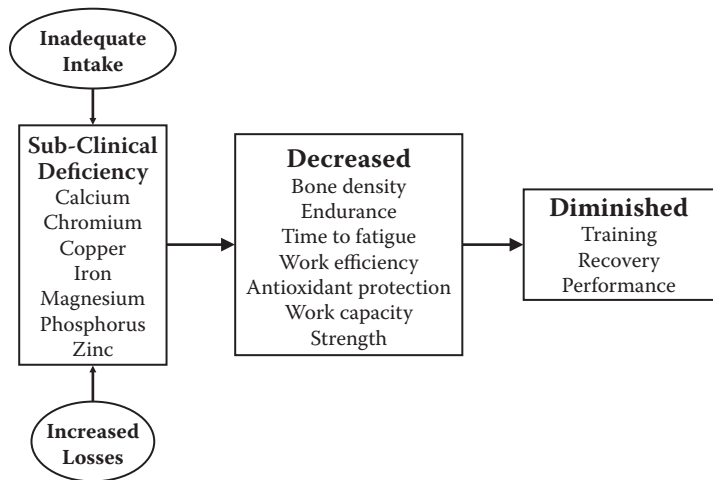


FIGURE 11.1 Summary of factors contributing to sub-clinical mineral element deficiencies and functional impairments leading to diminished capacity for physical performance.

also is a critical need to incorporate multiple markers, rather than reliance on a single marker, to identify subclinical deficiency because moderate nutrient deprivation can adversely affect several metabolic functions.

The most pressing issues center on the identification of valid and sensitive markers of subclinical deficiency of certain mineral elements. Although calcium plays pervasive roles in muscle function, assessments only focus on the calcium reserve in bone. Reliable indicators of magnesium and phosphorus status also are needed. Measures of cellular contents such as sublingual epithelial cells for magnesium and noninvasive determinations of phosphorylated energy intermediates (ADP/ATP, etc.) in skeletal muscle are opportunities to explore. Practical analytical and sophisticated instrumentation are available to meet this challenge. Assessment of chromium status requires a novel biochemical indicator that reflects tissue levels of chromium and cellular function. Although some measures of copper (superoxide dismutase activity) and zinc (serum/plasma zinc) have been found in some cases to discriminate physiological impairments, consensus is lacking. Utilization of advanced molecular and cell biology approaches that focus on measurements of gene expression (for example, protein and mRNA levels) should be encouraged in addressing the imminent need for reliable and sensitive indicators of subclinical mineral element deficiency in physically active people.

11.11 CONCLUSIONS

There is increasing evidence that many of the trace and macro elements have important roles in facilitating biochemical and physiological functions operational in the development and maintenance of physical work capacity and performance. One factor restricting further research on the interaction of mineral intake and physical activity has been the availability of valid indicators of subclinical mineral nutritional status. Recent findings indicate that two markers of magnesium and iron status merit incorporation in the biochemical assessment of mineral nutriture. Red blood cell magnesium is a surrogate marker for skeletal muscle magnesium and soluble serum transferrin receptor concentration is a biomarker of tissue iron depletion. Each of these nutritional markers is linked to perturbations in energy metabolism and impairments in work capacity and endurance. Although plasma zinc concentration is inversely related to muscle strength and endurance, its validity as a precise marker of zinc status remains controversial. The lack of acceptable indicators of calcium, copper, phosphorus, and chromium status hinders research to ascertain whether restricted or supplemental intakes affect biological processes (such as antioxidant protection, muscle function, and energy production) in response to physical training.

NOTE

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

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12 Assessment of Hydration of Athletes

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12.1 INTRODUCTION

Exercise associated with an increase in metabolic rate results in a subsequent rise in body temperature. Increases in skin blood flow and sweat production occur in response

to elevated body temperature as a means of dissipating heat. Sweat losses during vigorous exercise, particularly in warm to hot conditions, can be significant. If not adequately replaced, loss of body water (dehydration) can reduce exercise performance, decrease time to exhaustion, and increase heat storage.¹ A loss of 2% or greater of body mass can adversely affect exercise performance in both temperate and hot environments, particularly when the exercise duration is greater than 90 minutes.² With a loss of 3–5% of body weight, sweat production and skin blood flow decline, which can subsequently compromise physiological function (stroke volume, cardiac output, core temperature, and heart rate).³ This can lead to exertional heat illness, which ultimately can be fatal.^{4,5}

There is increasing evidence that water and electrolyte losses during exercise vary greatly between individuals.⁶ Consequently the American College of Sports Medicine position stand for fluid and electrolyte replacement recommends that hydration strategies should be prescribed on an individual basis.⁷ Hydration testing is an essential tool in the development of individual fluid intake guidelines, allowing the measurement of individual sweat rates and the adequacy of fluid and electrolyte replacement strategies. Common techniques used to monitor hydration status include body mass changes, urinary and hematological indices, salivary parameters, and total body water assessment. There is currently no gold-standard hydration assessment tool. Each method has both advantages and disadvantages relating to precision, accuracy, technical requirements, safety, cost, and convenience. Each of these factors should be taken into consideration when selecting the most appropriate tool for testing the hydration status of the athlete.

This chapter provides an overview of the impact of hypohydration, the various methods for assessing hydration status, factors that have an impact on rehydration, and current fluid intake guidelines. The chapter highlights the advantages and disadvantages of each hydration assessment technique and provides a guide for selecting the most appropriate tool for the individual athlete and the testing environment. Finally, there are practical recommendations in regard to application of the assessment techniques.

Throughout this chapter, the term “euhydration” refers to a state of fluid balance or normal body water content, that is, an adequate fluid intake to sustain normal urinary volume and concentration and relative stability of total body water (TBW), extracellular water, and intracellular water. “Hypohydration” and “hyperhydration” are defined as body water content deficits and excesses beyond normal fluctuations, while “dehydration” refers to the loss of body water as part of the process of becoming dehydrated. “Rehydration” is defined as the process of regaining body fluids from a dehydrated state toward euhydration.

12.2 IMPACT OF HYPOHYDRATION

12.2.1 THE EFFECTS OF HYPOHYDRATION ON THERMOREGULATORY AND CARDIOVASCULAR FUNCTION

Hypohydration occurs more rapidly in hot environments and during prolonged exercise.¹ Hypohydration reduces skin blood flow and sweating responses during exercise.⁸ Water lost through sweating originates from the intracellular (ICF) and extracellular fluid (ECF) compartments, and causes a reduction in plasma volume (hypovolemia).^{9–12} Reduced plasma volume can increase cardiovascular strain as

indicated by a reduced stroke volume and a consequent elevation in heart rate during exercise.^{11,13} This compromises the ability to dissipate heat from contracting muscles to the skin surface, thereby causing body temperature to rise, and increases the rate of body heat storage. At high ambient temperatures, evaporation of fluid from the respiratory tract and sweat from the skin surface provide the only means by which heat can be lost from the body.¹⁴ However, if the relative humidity is high, sweating contributes little to evaporative heat loss because high vapor pressure impairs evaporative cooling. Despite the relative inefficiency of sweating under these conditions, sweat formation continues, and the risk of heat injury increases.¹⁵

As dehydration progresses, there are negative effects on heart rate,^{11,16–18} stroke volume,^{11,13,19} cardiac output,^{11,13} fatigue,²⁰ skin blood flow,²¹ plasma volume,^{9,13,22} and rate of perceived exertion.^{17,23} The type of exercise can also contribute to the thermoregulatory response. Sporting activities with variable exercise intensities (that is, intermittent high-intensity exercise with short recovery bouts) have been shown to increase thermoregulatory (body temperature), cardiovascular (heart rate), and metabolic (lactate) stresses²⁴ when compared to steady-state exercise.

12.2.2 THE EFFECT OF HYPOHYDRATION ON EXERCISE PERFORMANCE

It is well known that even small body fluid deficits incurred before or during exercise can compromise performance^{16,23,25} well before they negatively affect health. The greater the body water deficit, the greater the physiological strain.¹³ Fluid losses in excess of 2% of body mass have been shown to have a substantial impact on physical and cognitive performance in a variety of climatic conditions.^{2,25,26} However, the critical level of fluid deficit that may have physical and cognitive performance implications varies from 1% to 10% of body mass loss.⁶ The magnitude of the performance deficit may vary according to variations in how performance is measured, environmental conditions, type of exercise, and individual characteristics such as differences in sweat rates and sweat composition.⁶ Nevertheless, it is widely known that the performance implications of hypohydration are exacerbated when undertaking prolonged exercise in the heat.²⁷ Physiological factors that impair exercise performance include increased core temperature, increased cardiovascular strain, increased glycogen utilization, and possibly altered central nervous system function.³ Impairment of physiological function and exercise performance caused by dehydration appear to be much greater when the fluid deficit is induced prior to²⁵ rather than during exercise.²³

Cognitive/mental performance such as complex skill execution and coordination as required in team sports have also been shown to be impaired by mild dehydration.^{17,28,29} Evidence suggests that fluid ingestion during exercise helps to attenuate the rise in heart rate and core temperature,¹⁶ delay the onset of fatigue,²⁰ prevent the deterioration of sport-specific skills,¹⁷ and ultimately increase time to exhaustion.²³

The means by which the body fluid loss occurs also determines the adverse effects experienced. For example, diuretic-induced dehydration results in a greater ratio of plasma volume loss to total body water loss compared with sweating.³⁰ However, exercise performance is not always impaired.³¹ Similarly, heat-induced dehydration results in a greater plasma volume reduction for a given body water deficit than

exercise-induced dehydration.³² It appears that there is a highly individual response as some athletes appear to tolerate hot conditions and dehydration, while others have to discontinue exercise.¹

It should be noted that while athletes might tolerate moderate fluid deficits in cool environments without significant impairment to exercise performance,³³ hypohydration is associated with serious health and physiological consequences and all attempts should be made to maintain physiological capacity.

12.2.3 HYDRATION AND HEALTH

Dehydration increases the risk of exertional heat illnesses such as heat cramps, heat exhaustion, and heat stroke that occur as a result of a significant rise in body temperature accompanying intense exercise. Predisposing factors leading to heat exhaustion and heat stroke include lack of heat acclimatization, inadequate training, medications, genetic predisposition, and viral illnesses that can potentially be fatal.³⁴ Athletes are at increased risk of developing exertional heat illnesses,³⁵ and in extreme cases rhabdomyolysis, a potentially life-threatening condition resulting from the breakdown of muscle fibers and the leakage of their contents into the circulation when exercising in a hypohydrated state.^{36,37} Hypohydration in combination with rhabdomyolysis (defined as serum creatine kinase greater than ten times normal) also appears to increase the risk of acute renal failure.¹

There may also be a relationship between exercise associated muscle cramps and high losses of both fluid and sodium, particularly in susceptible individuals who undertake prolonged strenuous exercise in the heat.^{38,39} Furthermore, hypohydration has been associated with reduced blood flow to the brain,⁴⁰ and altered intracranial volume.⁴¹

Conversely, overdrinking (drinking fluid volumes in excess of sweat losses) can result in exercise-associated hyponatremia, a condition characterized by low blood sodium concentrations. Plasma sodium levels below 125 mmol.L⁻¹ increase the risk of dilutional encephalopathy and pulmonary edema and result in symptoms of headache, vomiting, swollen hands and feet, fatigue, confusion, and disorientation, while plasma sodium levels below 120 mmol.L⁻¹ can be fatal.⁴² This has been shown to occur most commonly in smaller, nonelite individuals who run slowly, have lower sweat rates, and overdrink in competitive endurance events⁴² but has also been evident in sports where athletes are trying to prevent heat cramps and as a result drink excessive amounts of hypotonic fluids or water.^{42,43} To prevent hyponatremia occurring, Noakes⁴⁴ argues that athletes should drink according to thirst and not according to prescribed guidelines. An alternative strategy is to individualize recommendations on fluid intake based on sweat rate calculations (Section 12.4). Female athletes also appear to be more at risk of hyponatremia as they typically have lower sweat rates.⁴⁵

Age and gender differences also play a role on the impact of hypohydration on health. Females have lower sweat and electrolyte losses, while older adults have decreased thirst sensitivity and are slower to restore body fluid homeostasis due to reduced renal responses.⁴⁶ Children have lower sweat rates (less than 400 mL.h⁻¹) due to a smaller body surface area and faster rise in core temperature than adults.⁴⁷

12.2.4 CURRENT DRINKING PRACTICES OF ATHLETES

Although thirst is a sufficient stimulus for fluid intake under resting conditions,⁴⁸ athletes' drinking patterns are influenced by other factors such as habitual or social behaviors and recommended guidelines rather than physiological need.^{35,49,50} During exercise, however, dehydration usually occurs before fluid is consumed, demonstrating the inadequacy of thirst to stimulate sufficient drinking when fluid losses are high.^{48,51} This delay in voluntary fluid intake with the initiation of exercise has been termed "involuntary dehydration."⁴⁸ As a result, most athletes can expect to complete an exercise session with a mild to moderate degree of dehydration.

Athletes exposed to warm weather environments or hot/humid conditions can lose in excess of 3 L of sweat per hour, and can range from 0.5–2.0 L.hr⁻¹ and at best, replace only 30–70% of these losses.^{52–54} Euhydration can only be maintained during exercise if the rate of fluid ingestion and absorption equals the rate of fluid loss. Accordingly, some degree of hypohydration is likely during exercise because maximal sweat rates often exceed potential gastric emptying rates (greater than 1000 mL.h⁻¹).^{53,55,56} Although a reduction in body mass may appear to be of benefit in sports where power-to-weight ratio is important, there is evidence that modest dehydration does not provide an advantage of a reduced metabolic cost. Ebert and colleagues⁵⁷ demonstrated that uphill cycling performance in the heat was significantly compromised in athletes when hypohydrated by approximately 2 kg.

In many sports, particularly team sports, the opportunity to ingest fluids may be limited by the rules of competition, the number of breaks or substitutions in play, and fluid availability.^{53,58} For this reason, strategies such as pre-exercise hyperhydration may be warranted (see [Section 12.5.1](#)).

Many athletes start exercise hypohydrated due to attempts to make weight or inadequate time to fully rehydrate between sessions.⁷ Studies reporting the hydration status of athletes prior to elite-level competition have shown that hypohydration is prevalent among competitors.⁵⁹ Within weight-category sports, this scenario may be even more exacerbated where athletes may be "habitually hypohydrated" to assist with body mass management.⁶⁰ Despite acknowledgment of the importance of adequate rehydration strategies, many athletes fail to adhere to current sports nutrition guidelines for fluid replacement.^{37,53,60} Maintaining hydration status is a major challenge to the athlete, and particular efforts should be made to limit dehydration and the potential impairment to physiological function and health.

12.3 ASSESSMENT OF HYDRATION STATUS

An awareness of the performance implications of a compromise in hydration status has led to the pursuit of identifying tools that can be used to assess hydration status. Body mass changes,⁶¹ bioelectrical impedance analysis and dilution techniques,⁶² hematological indices (plasma osmolality, sodium, chloride, potassium, total plasma protein, blood-urea nitrogen, hematocrit, hemoglobin,⁶³ aldosterone,⁶⁴ and arginine vasopressin concentrations⁶²), urinary indices (osmolality, specific gravity, color, volume, sodium,⁶⁵ and conductivity^{66,67}), and salivary parameters (osmolality, total protein concentration, and flow rate)^{68,69} have all been trialed to assess hydration

status. A summary of the most common techniques available to assess hydration status, including their strengths and weaknesses, equipment requirements, and reference ranges, is presented in Table 12.1.^{77–79}

While plasma osmolality (OSM) has been cited as a criterion method for assessing hydration status,⁷⁰ the choice of marker will be influenced by the sensitivity and accuracy with which hydration status needs to be established,⁷¹ together with factors such as availability, expense, portability, invasiveness, time effectiveness, and technical expertise necessary to conduct procedures.^{72–74} preferred tool should be noninvasive,

TABLE 12.1
Hydration Assessment Techniques, Their Strengths and Weaknesses, Equipment Requirements, and Reference Ranges

Method	Advantages	Disadvantages	Equipment	Euhydrated Reference Range
Body mass	Simple, noninvasive, inexpensive, portable Able to self-monitor Immediate feedback Beneficial for estimating sweat rates when assessed pre- and postexercise, after accounting for during exercise nutrient intake, urine/ fecal losses, etc.	Does not measure body fluid absorption Requires knowledge of euhydrated mass Provides little information about presenting hydration status due to diurnal variations in body mass Limited to acute assessments across no more than 14 days	Professional quality digital scales, flat, nonabsorbent surface	< 1% variance in body mass from one day to the next
Plasma/serum osmolality	Precise, accurate Can detect acute dehydration	Requires trained phlebotomist Invasive, expensive Does not provide immediate feedback	Osmometer, centrifuge	< 290 mOsm.kg ⁻¹
Urine color	Simple, noninvasive, inexpensive Able to self-monitor Immediate feedback Portable	Influenced by diet, medications, illness, exercise, vitamins, acute ingestion of fluids	Urine color chart	Very pale yellow, pale yellow, straw-colored (< 3 on urine color chart although rarely 1)

TABLE 12.1 (continued)**Hydration Assessment Techniques, Their Strengths and Weaknesses, Equipment Requirements, and Reference Ranges**

Method	Advantages	Disadvantages	Equipment	Euhydrated Reference Range
Urine specific gravity	Noninvasive, convenient, inexpensive Immediate feedback Portable	Influenced by acute ingestion of fluids and presence of glucose and protein in urine Delayed response to acute dehydration	Refractometer (preferred) Dipsticks	< 1.020, although rarely below 1.010
Urine osmolality	Noninvasive Precise	Expensive Does not provide immediate feedback Delayed response to acute dehydration	Osmometer	< 700 mOsm.kg ⁻¹
Saliva	Simple, noninvasive	Flow rate less sensitive Possible effect of food and fluid intake on saliva flow rate Large interindividual variation Expensive if measuring osmolality	Polyester swab/tube, osmometer (osmolality), digital scales (volume), spectrophotometer (total protein)	< 61 mOsm.kg ⁻¹ although euhydrated reference range should be determined individually

Sources: Casa, D.J., Armstrong, L.E., Hillman, S.K., Montain, S.J., Reiff, R.V., Rich, B.S.E. et al., *J. Athl. Train.* 35, 212–24, 2000; Armstrong, L.E., Maresh, C.M., Castellani, J.W., Bergeron, M.F., Kenefick, R.W., LaGasse, K.E. et al., *Int. J. Sport Nutr.* 4, 265–79, 1994; Kovacs, E.M., Senden, J.M., and Brouns, F., *J. Sport Med. Phys. Fitness.* 39, 47–53, 1999; Shirreffs, S.M. and Maughan, R.J., *Med. Sci. Sports Exerc.* 30, 1598–1602, 1998; Walsh, N.P., Montague, J.C., Callow, N., and Rowlands, A.V., *Arch. Oral Biol.* 49, 149–54, 2004; Walsh, N.P., Laing, S.J., Oliver, S.J., Montague, J.C., Walters, R., and Bilzon, J.L., *Med. Sci. Sports Exerc.* 36, 1535–42, 2004; Oppliger, R.A., Magnes, S.A., Popowski, L.A., and Gisolfi, C.V., *Int. J. Sport Nutr. Exerc. Meth.* 15, 236–51, 2005; Oppliger, R.A. and Bartok, C., *Sports Med.* 32, 959–71, 2002; Armstrong, L.E., *Nutr. Rev.* 63 (6 Pt 2), S40–S54, 2005; Popowski, L.A., Oppliger, R.A., Patrick Lambert, G., Johnson, R.F., Kim Johnson, A., and Gisolfi, C.V., *Med. Sci. Sports Exerc.* 33, 747–53, 2001; Pagana, K.D. and Pagana, T.J., *Mosby's Diagnostic and Laboratory Test Reference*, 4th ed., Mosby Inc, St. Louis, Missouri, 1999; Casa, D.J., Armstrong, L.E., Ganio, M.S., and Yeargin, S.W., *Curr. Sports Med. Rep.* 4, 309–17, 2005; Oliver, S.J., Laing, S.J., Wilson, S., Bilzon, J.L., and Walsh, N.P., *Arch. Oral Biol.* 53, 975–80, 2008; Armstrong, L.E., Pumerantz, A.C., Fiola, K.A., Roti, M.W., Kavouras S.A., Casa, D.J., Maresh, C.M., *Int. J. Sport Nutr. Ex Metab.* 20, 145–153, 2010.

economical with minimal consumable requirements, technically simple, portable, valid, precise, and not influenced by factors unrelated to hydration status. Given this, urinary indices are generally the preferred method to assess hydration status, particularly in field situations, as urinary tests are simple, noninvasive, inexpensive, provide immediate feedback to the athlete, and are amenable to self-monitoring.^{72–73}

Periodic monitoring of hydration status creates an awareness of whether athletes are meeting their fluid intake needs and identifies those individuals most at risk of dehydration, such as those with high sweat rates or reluctant drinking behaviors.⁵⁸ Hydration assessments also complement the education process associated with good hydration strategies, providing feedback to athletes on how adjustments in drinking behavior influence hydration status. The assessment of hydration status has also become ingrained in the procedures used in the annual weight classification of athletes competing in weight category sports like wrestling.^{35,62,73,75} This has evolved as a result of athletes attempting to achieve rapid weight loss by aggressive dehydration practices to “make weight.”⁷⁶

12.3.1 BODY MASS CHANGES

It is assumed that body mass changes over short periods of time (that is, throughout an exercise session) or from one day to the next are due to water gained (food and fluid intake, substrate metabolism) or lost (via sweat and respiration) since no other body component can be gained or lost at such a rapid rate.⁸⁰ Supporting this assumption, it has been shown that changes in body mass and total body water generally move in the same direction and magnitude.^{62,81} Random variation in body mass from one day to the next is within the range of $\pm 1\%$.^{80,82} This information has been used to suggest a reduction in body mass in excess of this could be used in the field to indicate a state of hypohydration.

While day-to-day body mass changes may offer insight into acute changes in fluid balance, it cannot be used to accurately specify the state of hydration. This is because acute body mass changes merely indicate the degree of fluid deficit with reference to a baseline body mass measurement, which may not necessarily represent euhydration. As such, this tool should be used in conjunction with other hydration markers to confirm a baseline euhydrated body mass. Furthermore, body mass measurements should be made at the same time of day (preferably before breakfast or training but after voiding the bladder and bowel) and wearing minimal clothing,⁴⁹ so as to minimize the influence of factors other than hydration status that can have an impact on day-to-day variation in body mass. Other issues to consider include consistency in scales used,⁸³ menstrual cycle phase⁸⁴ in females, and chronic energy imbalance, which can influence body mass independent of hydration status.⁸⁵ As such, a euhydrated baseline body mass should not be used for any longer than 2 weeks.⁸⁰ The use of body mass change from one day to the next as an index of hydration status should not be confused with the acute measurement of body mass over an exercise session as part of procedures for measuring sweat rates.

12.3.2 URINARY INDICES

Urine is composed of a complex mixture of minerals, salts, and ionic and nonionic compounds, the relative proportions of which can vary markedly.^{86,87} Urea accounts for almost half the total solute composition of urine, followed by the major inorganic solutes of chloride, sodium, and potassium.⁸⁷ Other main constituents of urine include creatinine, uric acid, calcium, magnesium, phosphate, sulfate, and ammonia. The amber-yellow color of normal urine is due to the presence of several pigments, with urochrome being the predominant pigment.⁸⁸

With acute hypohydration, urine demonstrates acute changes in volume, color,^{66,89} specific gravity,^{72,89} osmolality,^{66,67,72} and conductance,^{66,67} creating an opportunity to assess hydration status via relatively noninvasive urinary indices.

12.3.2.1 Urine Specific Gravity

Urine specific gravity (Usg) is a measure of the density of a urine sample compared with the density of pure water.^{86,90} It is dependent on the molecular weight and number of particles (osmolality) in urine^{86,91} as well as the concentration of urea, protein, and glucose.^{73,91} As a result, the presence of heavy molecules, such as radiocontrast agents, and abnormal concentrations of protein and glucose can cause disproportionate increases in Usg with a minimal effect on urine osmolality (Uosm).^{86,91,92} Therefore, when present, these constituents may invalidate the use of Usg as an indicator of hydration status. Medical conditions in which Usg should not be used to assess hydration status are described in Table 12.2.⁹⁸ Similarly, athletes with large muscle mass may be incorrectly classified as hypohydrated and a higher usg cutoff may be warranted.⁹⁹

Several methods have been used to measure Usg, including hydrometry, refractometry, and reagent strips (dipsticks),^{73,86,90} although hydrometry is no longer considered

TABLE 12.2

Medical Conditions and Diagnostic Tests That May Affect Urine Specific Gravity Measurements

Condition/Test	Resultant Urinary Substance	Consequences
Diabetes mellitus with glucosuria	Glucose	Urine specific gravity underestimates total body water
Hyperparathyroidism/hypercalciuria	Calcium	
Salt-losing nephropathy	Abnormal urinary salts	
Nephrotic syndrome	Albumin	Urine specific gravity overestimates total body water
High dose of mannitol	Mannitol	
Use of radiocontrast media	Radiocontrast media	
Saline diuresis	Sodium	
Uremia	Urea	

Sources: Data from Chadha, V., Garg, U., and Alon, U.S., *Ped. Neph.* 16, 374–82, 2001; Voinescu, G.C., Shoemaker, M., Moore, H., Khanna, R., and Nolph, K.D., *Am. J. Med. Sci.* 323, 39–42, 2002; Bakhshandeh, S. and Morita, Y., *Michigan Med.* 74, 399–403, 1975.

a valid or reliable measuring device to determine Usg.⁹⁰ Refractometry is an indirect estimation of specific gravity by measurement of the urine's refractive index.⁸⁶ The amount of refraction is proportional to the number, mass, and chemical structure of the dissolved particles.⁸⁶ The refractometer requires only a small volume of urine,^{73,90,93} is temperature compensated,^{73,93} and can be used as a general guide to an athlete's hydration status.^{35,90} Furthermore, as refractometry is portable, noninvasive, inexpensive, objective, and simple to use by clinicians,^{73,75,90} it has become the preferred method for hydration assessment by many investigators.^{63,65,75,89,94,95} Manual hand-held units and digital refractometers are commercially available, with the manual unit being both cheaper and more robust. Both techniques provide almost identical results.

Research assessing the reliability and validity of using commercial reagent strips (dipsticks) to measure Usg has provided mixed results.^{90,93,96,97} Dipsticks lack the resolution of other techniques with graduations of 0.005 compared to 0.001 for refractometry.^{86,90} Consequently they are no longer considered an acceptable measuring device to determine Usg in wrestlers during minimal weight certification procedures due to concerns with accuracy, reliability, and precision.⁹⁷ Nevertheless, dipsticks offer a simple inexpensive method that athletes can use in the field to monitor substantial changes in hydration status.

12.3.2.2 Urine Osmolality

Osmolality is a measure of total urine solute concentration. Osmolality is measured using the freezing point depression technique on an osmometer, a laboratory-based piece of equipment that does not lend itself to being easily portable. Physiologically, urine osmolality (Uosm) is considered the most accurate measure of urine concentration.⁸⁶ This is because Uosm is less affected by solutes such as glucose, protein, and urea compared to Usg.^{86,91} Urine concentration is dependent on the presence of small solutes (for example, electrolytes, phosphate, urea, uric acid) and large solutes (such as protein, glucose)⁹³ and tends to increase with a reduction in urine volume. However, it should be noted that the composition and concentration of urine vary independently.¹⁰⁰ Therefore, when either large volumes of dilute urine are produced or when only small volumes of concentrated urine are produced, the total amount of solute excreted can be identical.¹⁰⁰

12.3.2.3 Urine Color

In an attempt to simplify urinalysis, Armstrong et al.⁶⁵ developed an eight-color scale including colors ranging from very pale yellow to brownish-green, which provides both clinicians and athletes a validated, simple, and inexpensive method to assess hydration status.³⁵ Using this scale, urine color (Ucol) has been shown to provide a reasonable index of hydration status in athletic or industrial settings when compared to Usg and Uosm,⁶⁵ at least when assessed by experienced personnel. However urine color can be influenced by an array of factors other than hydration status; details of this are specified in [Table 12.3](#).

Ucol should be assessed in a well-lit room with samples collected into inert, clear polypropylene containers and contrasted against the Ucol chart in the presence of a white background. It is not appropriate to assess Ucol from samples passed

TABLE 12.3**Common Foods/Drugs That May Affect Urine Color**

Common Foods/Drugs	Resultant Urine Color	Specific Test for Differential Diagnosis
Ibuprofen	Red/Red-brown	No specific test. Seek medication history
Anthraquinone laxative (Senna)	Yellow-brown	No specific test. Seek medication history
Riboflavin (vitamin B ₂) supplementation	Yellow	No specific test. Seek dietary supplementation/medication history
Carrots	Yellow-orange	Urine petroleum ether extraction/seek dietary history
Chlorophyll breath mints	Blue-green	No specific test. Seek dietary history
Rhubarb	Yellow-brown	No specific test. Seek dietary history
Beet	Yellow	No specific test. Seek dietary history

Source: Raymond, J.R. and Yarger, W.E., *South. Med. J.* 81, 837–41, 1988.

directly into a toilet. Finally, athletes may require training to assist in the accurate interpretation of hydration status via Ucol.

12.3.2.4 Urine Volume

Urine volume is another index that has the advantage of allowing athletes to independently monitor their own hydration status.⁷³ Athletes are often advised to monitor urine volume and frequency of urination when assessing their hydration status.¹⁰¹ This is particularly important during training camps and warm weather environments where significant fluid deficits can occur. However, urine volume is inconvenient to collect and assess,³⁵ necessitates compliance by the athlete,⁷³ is influenced by recent fluid intake, and provides little information about hydration status at a particular time point during the day.⁶⁵ In addition, subjectively monitoring daily urine frequency is only useful if the data collected can be compared to normal micturition patterns that have been established prior to competition, while assuming the athlete is in a euhydrated state.^{49,101} Urine samples reflect all urine that has collected in the bladder since the previous void, possibly explaining why urinary indices “lag behind” blood indices during acute changes in hydration status, as occurs throughout exercise.⁷⁰

12.3.2.5 Interpretation of Urinary Indices

The interpretation of urinary results can be complex because of the many factors that influence urine formation and composition. Factors that are known to influence urinary indices independent of hydration status include attempted rapid rehydration with hypotonic fluids,⁶⁶ severe dehydration,⁷⁷ intravenous saline infusion,¹⁰² and the consumption of caffeine¹⁰³ and alcohol.¹⁰⁴ Moreover, the presence of normal or abnormal urinary constituents, certain disease states, diet (beets, carrots), temperature, and pH can also influence urinary indices of hydration status, the magnitude of which varies with the technique.^{86,105} Storage of urine samples between the point of micturition and analysis can also influence results, with sample analysis recommended within 30–60 minutes.¹⁰⁶

When sample analyses cannot be undertaken within this time frame, they should be refrigerated as soon as possible after micturition at 2–8°C. However this may promote crystal formation, rendering samples invalid for assessment via Ucol.¹⁰⁶

Urinary values may vary significantly with certain medications (for example, antibiotics, diuretics), supplements, fever, endocrine function, metabolism, exercise, and the presence of bacteria.^{77,87} Furthermore, atypical dietary patterns such as strict vegetarianism^{86,107} and exclusive milk consumption in children can alter urinary solute composition and affect both Usg and Uosm results.^{86,105,107} Usg specificity is also reduced in athletes with large muscle mass due to an increase in urine protein metabolites.⁹⁹ When attempting to undertake hydration assessments, it is important to be aware of the potential limitations of each technique before selecting an assessment tool.

Ucol, Uosm, and specific electrical conductance may be poor indicators of hydration status in athletes during the first 6 hours after exercise-induced hypohydration (3% body mass loss) as a result of rapid *ad libitum* rehydration.⁶⁶ Athletes who attempt to aggressively rehydrate with large volumes of hypotonic fluids during a brief period will produce large volumes of dilute, pale-colored urine even if the body is still in a state of dehydration.^{66,108} Excessive urine production can occur before total body fluid deficits have been completely replenished in the intracellular and extracellular spaces, thus masking hydration status. Fluid ingestion can temporarily produce a urine sample that does not accurately reflect hydration status.^{72,108,109} Consequently, urinalysis is best undertaken on upon-waking samples, eliminating the influence of acute food and fluid intake.^{65,67} Despite this, there may still be value in “spot checks” at other time points during the day to confirm a state of dehydration given that while false negatives are possible (hypohydrated yet urine sample indicates a state of euhydration), false positives (euhydrated yet urine sample indicates a state of dehydration) are unlikely at any stage during the day for the majority of athletes.

In summary, Ucol, Usg, Uosm, and urine volume can be used either independently or simultaneously to assess an athlete’s hydration status. While Uosm may be considered the preferred urinary index of hydration status within a clinical environment where elevations in urinary solute load are possible independent of hydration status (for example, presence or elevation of glucose, protein and urea in urine), healthy athletes do not require such a precise and sophisticated measurement.⁶⁷ Consequently monitoring Ucol or Usg provides an inexpensive, noninvasive, practical, and effective method of assessing hydration status that can be easily used by athletes.^{35,73}

12.3.3 HEMATOLOGICAL INDICES

Numerous blood-borne indices have been used to assess hydration status. Changes in hemoglobin and hematocrit concentrations may be used to assess intra- and extracellular fluid shifts provided baseline/euhydrated values are established.^{9,71,73,95,110} However, to ensure comparable results, a standardized posture must be assumed for about 15–20 minutes^{89,95,101,110} to remove the influence of posture on fluid compartment shifts.⁸⁷ If baseline values are known, relative changes in plasma volume can be estimated from changes in hematocrit and hemoglobin concentrations.¹¹¹

In order for markers of hydration status to be of practical use, they must be capable of detecting a body water deficit of 2–3% of body mass.¹⁰¹ Indeed, plasma osmolality

is sensitive enough to identify acute body fluid deficits of as little as 1–5% body mass, as can occur during exercise.^{70,72} Furthermore, unlike urinary indices, there does not appear to be a delayed response to the effect of hypohydration on plasma osmolality. However, changes in plasma osmolality in response to moderate hypohydration (< 3% of body mass) may dissipate over time as plasma volume is defended to maintain cardiovascular stability.⁹⁵

While the immediate measurement of osmolality is preferred, serum and plasma samples can be left at room temperature for upwards of 3 days without influencing osmolality data.¹¹² Despite this, the measurement of blood parameters in the field is impractical, requires a qualified phlebotomist, and is expensive and invasive.⁷³ In addition, blood collection can cause discomfort to the athlete and introduces the risk of infection, bruising, and vein damage.^{73,101} Clearly, these limitations restrict the use of this method to regularly monitor hydration status. Such tests may be considered more appropriate in research and clinical settings where reliability, accuracy, and precision are essential.

12.3.4 SALIVARY PARAMETERS

Recently, salivary parameters have been identified as potential indices of hydration status during progressive acute hypohydration to 3% body mass loss.^{68,69,79} Strong correlations between percent body mass loss and saliva osmolality and total protein concentration were found (mean $r = 0.94$ and 0.97 , respectively; $P < 0.01$).⁶⁸ However, changes in saliva flow rate were less sensitive to changes in hydration status measured by percent body mass loss (mean $r = -0.88$; $P < 0.01$)⁶⁸ and urine and plasma osmolality.⁶⁹

Compared with blood collection procedures, collecting saliva samples using a polyester swab is simple, noninvasive, and inexpensive.⁶⁸ Nonetheless, other factors may influence the validity of saliva parameters as a potential marker of hydration status, including a possible short-term effect of food and fluid ingestion on saliva flow rate. There is a need for further research to elucidate the mechanisms responsible for salivary responses during progressive acute hypohydration and to determine the sensitivity of salivary parameters in a field setting.⁶⁸

12.3.5 TOTAL BODY WATER ASSESSMENT

Biochemical parameters and body mass changes are indirect markers of hydration status and do not indicate total body water (TBW) change.⁸² Measuring TBW by dilution techniques such as deuterium oxide^{62,110,113,114} and bioelectrical impedance analysis or spectroscopy allows the determination/estimation of TBW content.⁶² Total body water levels can be measured by administering an oral dose of an isotope tracer such as deuterium oxide ($^2\text{H}_2\text{O}$ or D_2O) and measuring the isotope enrichment in saliva, urine, or serum samples.^{115,116} The dilution technique assumes the distribution and diffusion of the isotope tracer in the body fluid compartments are comparable to the distribution and diffusion of water.¹¹⁵ Deuterium dilution has been the preferred technique^{62,65,110,113,117} and has served as the gold standard to validate blood and urine indices of hydration status¹¹⁰ and bioimpedance spectroscopy analyses.¹¹⁸ With careful attention to administration and measurement procedures, TBW can be determined by isotope dilution with a precision of 1–2%.^{114,115} Despite its small

measurement error,¹¹⁴ TBW measurements are generally restricted to laboratory-based assessments, as they are time consuming and expensive. In addition, an equilibrium period of 3 to 4 hours is required after dose administration, without food, fluid, or exercise,^{114,115} making it inadequate to track acute changes in TBW.

Bioelectrical impedance analysis and spectroscopy use electrical current to estimate TBW content. Electrical current passing through the human body is resisted by body tissues and water. This technique represents a noninvasive, safe, and relatively easy means to assess TBW.^{73,119,120} However, the accuracy and reliability of impedance testing requires strictly standardized and controlled conditions, particularly electrode placement, subject position, recent exercise, previous food and fluid intake, hydration status, skin temperature,^{121,122} and plasma tonicity.¹²⁰ Even when these conditions are achieved, bioelectrical impedance analysis is only able to identify half of acute changes in fluid balance.¹²³ Estimates of TBW may also be compromised if the prediction equations used are not applicable to the specific individual or population under investigation.¹²¹ As such, the current consensus is that bioelectrical impedance analysis can provide an indication of total body water but lacks the precision, accuracy, and resolution for monitoring small changes in hydration status, as occur within the athletic setting.^{73,78,118,120}

In summary, impedance and dilution techniques appear to be of limited practical use to monitor the hydration status of athletes. This is mainly because athletes are commonly in an “uncontrolled” or postexercise state.¹¹⁹ Although dilution techniques are considered the gold standard for estimating TBW, the analytical requirements, expense, and degree of control required limit the routine use of this technique for hydration assessment.

12.4 ASSESSMENT OF SWEAT RATE AND COMPOSITION

12.4.1 SWEAT RATE ASSESSMENT

Assessment of an athlete’s individual sweat rate is a practical way to obtain information about his or her current hydration status and can assist with prescription of individual fluid requirements. Sweat rate assessment requires the use of two measurements: (1) upon-waking Usg and (2) fluid intake and body mass changes during exercise. The equipment needed to conduct sweat rate assessment is listed in Table 12.4.

TABLE 12.4
Equipment Checklist for Sweat Rate Assessment

Urine Specific Gravity	Body Mass Changes
Portable refractometer	Scale accurate to 100 g for body mass
Sample jars for urine	Scale accurate to 5 g for bottle/food weight
Pipettes	Towels for wiping down athletes
Plastic gloves	Board to place scales on
Sterile wipes	Weather monitor for temperature and humidity
Water (sterile or tap)	Chart to record data
Plastic covering for table	Stopwatch/clock

The following procedure describes the steps required to conduct a sweat rate assessment.

1. Pre-exercise:
 - The athlete presents with upon-waking urine sample to assess hydration status pre-exercise.
 - Refractometer Usg test is conducted. Ensure the refractometer is zeroed with distilled water. Pipette a sample of at least 1 mL of urine onto the reading surface and record result. Wipe the reading area clean with a sterile wipe and repeat.
 - Record the air temperature and relative humidity.
 - Weigh the athlete in minimal clothing with bladder voided. Record any fluid consumed in the 30 minutes prior to being weighed.
 - Weigh drink bottles that are to be consumed during the exercise session. Record the type of fluid in each bottle.
 - Take note of the time the exercise session commences.
2. During exercise:
 - Record a second reading of air temperature and humidity.
 - Weigh each drink bottle as it is emptied and reweigh if refilled.
 - Note the number and timing of drink breaks.
 - If the athlete visits the bathroom, ensure that he or she is weighed before and after emptying bladder. Alternatively, collect urine and weigh.
3. Postexercise:
 - Record the finish time of the exercise session.
 - Ask athlete to wipe off any residual sweat from his or her body, hair, and clothing. Record the weight of the athlete in identical clothing to pre-exercise weight.
 - Ask the athlete to empty his or her bladder (if possible) and record the post-bladder voided weight.
 - Reweigh any remaining drink bottles and unconsumed food with packaging.
 - Record a final reading of air temperature and humidity. Calculate an average of all three readings.

Practitioners can enter the results obtained from the sweat rate assessment into the data entry sheet provided in [Table 12.5](#).

This data can be used to calculate sweat rate and obtain information on fluid intake and drinking behaviors during exercise. The calculations required to determine sweat rate are as follows:

1. Fluid intake (absolute) = (fluid 1 + fluid 2 + fluid 3) mL
2. Fluid intake (relative) = [absolute fluid (mL) / exercise time (min)] \times 60 mL/hr
3. Weight loss (kg) = Pre-exercise weight – postexercise bladder voided weight
4. Urine loss (mL) = weight pre-bladder void – weight post-bladder void
5. Sweat losses (absolute) = (Weight loss + fluid intake – urine loss) mL
6. Sweat rate = [absolute sweat loss (mL) / exercise time (min)] \times 60 mL/hr

7. Percent fluid replaced (%) = [fluid intake (mL) / sweat loss (mL)] × 100

8. Percent body weight change (%) = (weight loss/pre-exercise weight) × 100

Sweat rate calculations are based the assumption that 1 g of weight loss is equal to 1 mL of fluid, and the majority of the fluid loss is sweat. It does not account for respiratory and metabolic water or fuel losses. In distance events such as marathons, weight

TABLE 12.5

Sweat Rate Assessment Data Entry

Name:		Sport:	
Test day USG:	Euhydrated: Yes / No	Bladder voided: Yes / No	Comments:
Conditions			
Reading 1 (pre)	Temperature:	Humidity (%):	Average:
Reading 2 (during)	Temperature:	Humidity (%):	
Reading 3 (post)	Temperature:	Humidity (%):	
Training			
Type:	Start time:	Finish time:	Total time =
Weight			
	A. Pre-exercise wt (kg):	B. Postexercise wt (kg):	Postexercise bladder void (kg):
During exercise bladder void Time:	A. Pre-bladder void wt (kg):	B. Post-bladder void wt (kg):	Urine volume (A – B) =
Fluid			
Bottle 1 Type fluid:	A. Weight pre:	B. Weight post:	Fluid 1: A – B =
Bottle 2 Type fluid:	A. Weight pre:	B. Weight post:	Fluid 2: A – B =
Bottle 3 Type fluid:	A. Weight pre:	B. Weight post:	Fluid 3: A – B =
Drink breaks (mark off)	Time	Comments	
	1		
	2		
	3		
	4		
	5		
	6		
	7		
	8		
	9		
Food			
Type =	A. Weight food pre (g)=	B. Weight food post (g) =	A – B =

loss from other sources may account for 1.5 to 2 kg of weight loss.¹²⁴ Nevertheless, sweat rate assessment of the athlete can assist with the development of individual fluid replacement guidelines. The guidelines should consist of advice on estimated fluid requirements before, during, and after exercise as well as goals and strategies for fluid replacement over the remainder of the day. Information on the most appropriate type of fluid and the volume and timing of intake can also be addressed. [Table 12.6](#) provides a template for a sweat rate assessment report that can be provided to athletes to complement advice on specific strategies to improve drinking practices.¹²⁵ Feedback should take into account factors that are relevant to the individual athlete and his or her sport (such as taste preferences, opportunities to consume fluid, rules of the sport, playing position in team sports). Specific recommendations for fluid intake pre-, during, and post-exercise are provided in [Section 12.5](#).

Although there is a need for individualized sweat rate assessment and fluid replacement guidelines, estimating sweat losses can assist with planning needs for sporting populations and military personnel, particularly when exposed to heat stress. Sweat rate can be predicted from a range of parameters, including the environmental conditions, metabolic rate, and clothing. The original Shapiro prediction equation,¹²⁶ formulated 25 years ago, was based on laboratory experiments that determined energy expenditure of men at rest and up to moderate exercise intensities over a range of environmental conditions while wearing a range of clothing.¹²⁶ This equation has been used to determine the U.S. dietary reference intakes (DRI) standards for water and electrolytes.¹²⁷ More recently this equation has been shown to overpredict sweat

TABLE 12.6
Sweat Rate Assessment Report

Name:	Date:
Sport:	Training session :
	Duration:
Environmental conditions	Temperature:
	Humidity (%):
Urine specific gravity: (A reading < 1.020 is considered well hydrated)	
Sweat losses:	Total (mL) =
	Hourly loss (mL/hr) =
Urinary losses	Urine losses
	Total (mL) =
Total fluid loss	Total (mL)
	Hourly loss (mL/hr) =
Fluid intake	Total (mL) =
	Hourly intake (estimate) (mL/hr) =
Fluid replacement	% of your sweat loss
Comments	

Source: Adapted from Gatorade Sports Science Institute, Australian Institute of Sport, and Sports Dietitians Australia, 2006.

rates, resulting in a new prediction equation that more accurately estimates sweat losses.¹²⁸ As previously suggested, there may be specific situations where use of this equation is warranted. However, the preferred option for the practitioner is to conduct a sweat rate assessment as outlined above.

12.4.2 SWEAT COMPOSITION ASSESSMENT

Knowledge of sweat composition can complement sweat rate assessment and assist with development of fluid intake guidelines. Sodium and chloride are the primary electrolytes lost in sweat, with calcium, magnesium, and potassium present in smaller amounts.¹²⁹ There is a large individual variability in the concentration of electrolytes in sweat¹³⁰ with sodium and potassium concentrations ranging from 20 to 80 mmol.L⁻¹ and 4 to 8 mmol.L⁻¹, respectively.^{130,131} Profuse sweating can lead to significant fluid and electrolyte loss, and potentially results in exercise-associated hyponatremia during endurance activity.¹³¹ However, higher sodium intakes have been associated with elevated blood pressure and cardiovascular risk,¹³² and therefore it would be unwise to recommend that all athletes consume a high-sodium diet. Rather, specific prescription of sufficient sodium to replace losses experienced during exercise in the postexercise period will assist with aggressive rehydration while avoiding any of the complications associated with a habitually high dietary sodium intake. Sweat composition analysis can help to identify athletes who have a high concentration of electrolytes in their sweat, allowing individual prescription of sodium and other electrolytes.

Two methods are used to assess sweat composition: whole-body wash down and regional skin surface collection (sweat patches).¹³⁰ A comparison of the two methods is shown in Table 12.7. Whole-body wash down is considered the criterion method to determine electrolyte losses because all sweat runoff is collected and accounted for. Sweat patch analysis involves collecting sweat from a number of locations on the body and using these results to predict whole-body sweat composition (Table 12.8). This method must be conducted in conjunction with the sweat rate assessment described above. Although this is a practical approach to determining sweat composition in the

TABLE 12.7
Comparison of Sweat Composition Analysis Methods

	Sweat Composition Analysis Technique	
	Whole-Body Wash Down	Sweat Patches
Testing environment	Laboratory	Laboratory or field
Exercise modality	Cycling	All sports
Accuracy	Criterion method	Use of equation weighted for
	All sweat accounted for	local sweat rate and body surface area
Degree of difficulty	Complex and impractical	Simple and practical

Source: Sports Dietitians Australia, Fact Sheet, *Sports Drinks*, Sports Dietitians Australia, Melbourne, 2009.

TABLE 12.8
Sweat Patch Technique

Step	Method
1	Clean skin with deionized water, wiping dry with sterile gauze
2	Apply an absorbent sterile patch pad to a specified body parts (e.g., forearm) ensuring that the patch adheres to the skin
3	After exercise, carefully remove patch with dry forceps and place into a small (30 mL) sealed container (centrifuge tube), ensuring that no sweat evaporates from the patch after removal.
4	The sweat can be removed by centrifugation and the concentration of electrolytes determined using flame photometry, ion chromatography, or ion-selective electrode. ¹³¹ To work out sweat sodium losses, combine results with sweat rate calculations.

field, it has been shown to generally overestimate sodium and potassium sweat concentration.¹³³ The sites most commonly used include the forehead, forearm, chest, back, thigh, and calf.⁶ Patterson et al.¹³⁴ found that sodium collections from the calf and thigh were the most closely correlated to the whole-body wash down technique. However, Baker et al.¹³³ found the thigh and chest to be the best sites in both males and females. It is likely that a large number of sites will enhance reliability of sweat patch techniques, but this needs to be balanced against the additional cost associated with multiple site analysis. Equations are available to predict whole-body sweat electrolyte losses from regional skin surface collections¹³³ with a single site (forearm) often used. While the validity of regional skin surface collections as a direct measure of whole-body sweat electrolyte concentration remains debatable,¹³⁵ its application in the field across a broad range of exercise modalities ensures it remains an attractive option for practitioners.

The interpretation of sweat composition assessment should be considered along with the results of sweat rate, fluid intake, level of dehydration, exercise intensity and duration, and environmental conditions. Within a normal population, sweat sodium will range from 10–80 mmol/L and potassium from 4 to 8 mmol/L. Values outside of this range are likely to represent an error in the collection technique. However, individuals with cystic fibrosis will have higher concentrations of sweat sodium, and there are a few rare genetic conditions where the value may fall outside this range. Heat acclimatization improves the ability to reabsorb sodium and chloride, resulting in lower sweat sodium concentrations.¹³⁶

12.5 RECOMMENDATIONS FOR FLUID INTAKE

Euhydration is particularly important to the athlete when there is short recovery between sessions, there are multiple competition events during the day, or the athlete is identified as being hypohydrated in the morning prior to training. In each of these circumstances, it is worth considering the various factors that influence the rate of rehydration, including beverage composition, temperature, and rate of consumption. Recent studies have shown that the rate of fluid consumption is one of the key factors in successful rehydration.^{10,137} Ingestion of large volumes of fluid in a short space of time leads to greater diuresis compared to when the volume is consumed over a

longer time span.^{10,138} The electrolyte content, palatability, and temperature of the beverage also play a role.¹³⁹ Furthermore, the rate of fluid absorption needs to be considered when rapid rehydration is imperative to performance during exercise, particularly in the heat. Factors that have an impact on the rate of fluid availability include the speed of gastric emptying, the intestinal absorption, and retention of the fluid within the intra- and extracellular space.^{15,140} The following section outlines the current recommendations on fluid intake before, during, and post-exercise and the various factors that influence fluid availability.

12.5.1 PRE-EXERCISE HYDRATION

Athletes should aim to start exercise in a euhydrated state with minimal gastrointestinal discomfort. If an athlete has consumed adequate fluid throughout the day and sufficient time has elapsed since the last exercise bout, he or she is more likely to be euhydrated. However, if there have been substantial fluid deficits and the individual has not had adequate time to replace losses, then more aggressive pre-exercise hydration strategies may be required. There is currently no consensus on the optimal pre-exercise hydration strategies for athletes. The American College of Sports Medicine recommends consuming beverages 4 hours before the exercise bout at around 5–7 mL.kg⁻¹. If the individual does not produce urine or it is very concentrated, then 3–5 mL.kg⁻¹ should be consumed around 2 hours before the event.⁷ Consuming beverages or food containing sodium may offer some advantage as it will stimulate thirst, reduce urine output, and thus promote greater retention of ingested fluids. As rehydration during exercise depends upon maximizing the rate of delivery from the stomach to the intestine, there is an advantage in starting exercise with a comfortable volume of fluid in the stomach.¹⁴¹ Distension of the stomach will assist with gastric emptying provided that fluid is consumed periodically once exercise commences.

Pre-exercise sodium loading has been investigated as a means of hyperhydrating prior to exercise. There is evidence that consuming beverages containing higher levels of sodium than standard sports drinks (164 mmol Na L⁻¹) consumed approximately 1.5 hours before exercise can lead to increased plasma volume and exercise capacity in the heat in both men and women.^{142,143} However, it is currently unclear whether this is related to cardiovascular or thermoregulatory mechanisms. Beverage temperature can also lead to an improvement in exercise capacity in the heat. Ingestion of cold fluids (4°C) before (or during) exercise has been shown to improve performance and attenuate the increase in core temperature that occurs in a hot environment.¹⁴⁴ Precooling techniques, such as whole-body precooling,¹⁴⁵ have previously been used in athletes undertaking endurance exercise in the heat to lessen the strain on the thermoregulatory system. The ingestion of cold beverages may provide a more practical and comfortable approach that can lead to increased endurance capacity by assisting with the management of core temperature.

12.5.2 FLUID REPLACEMENT DURING EXERCISE

The main aim of drinking during exercise is to maintain physiological capacity and minimize any risk of exertional heat illness. The factors that have an impact on fluid

availability include the rate of gastric emptying, intestinal absorption, and retention of the fluid within the intra- and extracellular space.^{15,140} Maximizing the rate of gastric emptying and delivery to the intestines is most important during exercise in the heat when rapid delivery of fluid is required. Gastric emptying has been shown to be influenced predominantly by the energy density and also the osmolality of the fluid.¹⁴⁶ Most sports drinks are formulated to have an osmolality close to body fluids; however, a drink that is slightly hypotonic (dilute glucose-electrolyte solutions) may result in a greater rate of water uptake due to the osmotic gradient created between the intestinal contents and the intracellular fluid in the cells of the intestinal wall.¹⁴⁷ As carbohydrate intake at a rate of 30–60 g.hr⁻¹ (approximately 1 L of sports drink) has been shown to benefit performance through sustained exercise intensity,¹⁴⁸ and is independent and additive to water alone,¹⁶ there is merit in adding moderate amounts of carbohydrate to sports drinks. Therefore, if the primary concern of the exercise bout is to provide energy, and dehydration is of less concern, then addition of carbohydrate as an exogenous fuel source is warranted. There is also some evidence that there is a greater rate of carbohydrate delivery and improved endurance performance with the consumption of multiple transportable carbohydrates (for example, glucose, sucrose, maltodextrin, and fructose) compared with a single carbohydrate source.^{149,150}

Many factors influence the degree of fluid and electrolyte losses during exercise such as ambient temperature, clothing, humidity, heat acclimatization, training status, and genetic predisposition.⁷ Relying on thirst alone may not give a true indication of actual needs and may lead to dehydration, whereas overdrinking can result in hyponatremia, which can have potentially fatal consequences.⁴² There is also evidence that athletes may experience gastrointestinal discomfort if they attempt to replace fluid at a rate that matches sweat loss.¹⁵¹ Daries and colleagues¹⁵¹ demonstrated that fluid consumption at a rate of approximately 330 mL every 15–20 minutes (1.0 to 1.4 L) over 2 hours of running in a thermoneutral environment did not improve performance and resulted in abdominal discomfort in most subjects. However, there is evidence that athletes can “train” themselves to tolerate larger fluid volumes. Lambert and colleagues¹⁵² demonstrated that trained runners could better tolerate drinking an isotonic carbohydrate-electrolyte drink that nearly matched their sweat rate when they consumed the same volume over repeated sessions in a moderate environment. This was not related to an improvement in gastric emptying. Although the mechanism remains unclear, it has been proposed that this is related to gastric distension.¹⁵³

All athletes should develop their own hydration strategy for fluid replacement during exercise based on assessment of sweat rate and fluid balance in a variety of training and competition situations and environmental conditions.^{6,153} This will help the athlete to determine whether a more aggressive approach to hydration is required. Athletes should drink as much as practically and comfortably possible, and attempt to consume around 70–80% of fluid losses but never more than their sweat losses. The athlete will need to be advised on the volume of fluid he or she needs to consume, the type of fluid, the timing of intake based on the opportunities he or she has to drink during the activity, and gastrointestinal comfort. In team sports, the athlete must take opportunities such as quarter/half time, pauses in play, timeouts, and substitutions to access fluid.

Some examples of the composition of different types of fluids are provided in [Table 12.9](#).¹⁵⁴

TABLE 12.9
Composition of Different Types of Fluids

Fluid	Carbohydrate (%, Type)	Sodium (mg per 100 mL)	Comments
Water	Nil	3–5	Suitable for short-duration activity; assists with meeting hydration needs; No carbohydrate or electrolyte replacement.
Sports drinks	4–8 sucrose, glucose	20–60 (10–25 mmol.L ⁻¹)	Best choice during exercise; moderate electrolytes; meets fluid and fuel needs
Soft drink	11 sucrose	10	Slowly absorbed, negligible electrolytes; cola provides caffeine; flavor alternative in long events
Fruit juice	8–12 fructose, glucose	7	Slowly absorbed; negligible electrolytes; may cause gastrointestinal (GI) upset due to high fructose load
Sports water	0–4% sucrose	0–12	Negligible electrolytes; addition of vitamins and herbs; may be suitable for short-duration activity
Energy drink	10–13 Sucrose, glucose	10–120	May contain caffeine, taurine, guarana, vitamins, herbs or other additives; flavor alternative in long events

Source: Adapted from Sports Dietitians Australia, Fact Sheet, *Sports Drinks*, Sports Dietitians Australia, Melbourne, Australia, 2009.

12.5.3 POSTEXERCISE FLUID REPLACEMENT

The goal of postexercise fluid replacement is to restore fluid and electrolyte deficits. If recovery time is sufficient, consumption of water and normal meals will restore euhydration.⁷ However, if recovery time is short and dehydration is substantial, more aggressive strategies are warranted. The main factors influencing the postexercise rehydration process are the volume and sodium content of the ingested fluid.^{108,155} Obligatory sweat, respiratory, metabolic, and urinary losses persist throughout the recovery phase, even in the hypohydrated state.¹⁰³ Therefore, a volume corresponding to 150% of the fluid deficit must be ingested to acutely restore fluid balance (i.e., 1.5 L for every kilogram lost).¹⁰⁸

The inclusion of sodium in oral rehydration solutions is recommended since it is lost in sweat and should be replaced for rapid restoration of fluid balance.^{155–157} Sodium, in combination with carbohydrate, improves palatability while also stimulating the osmotically dependent dipsogenic factors that increase voluntary fluid intake.^{139,156,159} Moderately high sodium intakes (50–60 mmol.L⁻¹) to levels found above those in sports drinks (10–25 mmol.L⁻¹) are justified when effective fluid replacement must be achieved within several hours after completing exercise.^{108,159} A study that evaluated the effect of varying levels of drink sodium content (2, 26,

52, or 100 mmol.L⁻¹) on the rehydration process found that the subjects who consumed the two higher-sodium beverages were euhydrated 5.5 hours after the end of the rehydration period.¹⁵⁵ Despite the ingestion of a drink volume equivalent to 1.5 times their estimated sweat losses, subjects were in net-negative fluid balance after the consumption of the two low-sodium fluids. Cumulative urine output was inversely related to the sodium concentration of the ingested fluid. Furthermore, a study by Nose and colleagues¹⁵⁶ found a delay in rehydration following the ingestion of plain water and attributed this to the removal of the osmotic drive to drink and a rise in free water clearance. Thus, unless the rehydration beverage contains sufficient sodium, fluid intake will merely increase urinary output, delaying full restoration of fluid balance.^{101,108,158}

To promote rehydration and correct fluid deficits, athletes are encouraged to consume a combination of sodium-containing foods and fluids (Table 12.10), which have the potential to decrease cumulative urine output.^{155,160} Despite this, solid food is not always appealing to athletes following strenuous exercise as a result of gastrointestinal discomfort¹⁰⁹ and optimal rehydration beverages will be just as effective and convenient. However, consumption of meals may be necessary to ensure full hydration is ultimately achieved,¹⁰⁸ as solid food provides additional fluid and electrolytes, and assists with fluid retention.¹⁶¹

Consumption of skim milk has been shown to effectively restore fluid balance postexercise.¹⁶² This has been attributed to its relatively large quantities of electrolytes (Na⁺ 32 mmol/L, K⁺ 42 mmol/L and Cl⁻ 36 mmol/L) in comparison to sports drinks, resulting in decreased urine production. Furthermore, milk can provide both carbohydrate and protein, which can contribute to restoration of muscle glycogen stores. However, milk is not a suitable alternative for athletes who are lactose intolerant. Alcohol can act as a diuretic and increase fluid output, and therefore should be

TABLE 12.10
Sodium Content of Common Fluid and Foods
for Athletes Who Have High Sodium Losses

Food/ fluid	mg per Serve
Sports drinks (4 cups : 1 liter)	~ 330
Milk (4 cups : 1 liter)	457
Bread (2 slices : 80 g)	249
Corn Flakes cereal (1 cup : 30 g)	320
Noodles (1 cup cooked : 170 g)	434
Cheese (2 slices : 60 g)	390
Ham (1 slice : 20 g)	325
Salmon, tinned (2 tabs : 40 g)	211
Olives (10 medium : 40 g)	387
Tomato juice (1 cup : 250 mL)	750

Source: Adapted from Maughan, R.J. and Shirreffs, S.M., *Int. J. Sport Nutr. Exerc. Meth.* 18, 457–72, 2008.

consumed in moderation during postexercise recovery.¹⁰⁴ Caffeine is a common additive in many beverages and some sports foods. It has been implicated as potentially increasing urine output and contributing to dehydration.⁷ It appears that small doses of caffeine are unlikely to increase urine output and cause dehydration,¹⁶³ although this has not been tested during exercise or in already hypohydrated individuals.

The value of including other electrolytes in sports drinks is unclear.¹⁶⁴ Potassium may be necessary for intracellular fluid compartment restoration; however, further investigation is required to provide conclusive evidence.¹¹

Individual fluid replacement protocols should be implemented considering the athlete's sweat rate, exercise intensity, nature of the sport, recovery time between exercise bouts, and environmental conditions to ensure optimal recovery and maintenance of exercise capacity.³⁵

The impact of the carbohydrate content and osmolality of fluid consumed postexercise appears to be of less importance when the primary aim is to restore fluid balance. In a recent study by Evans et al.,¹³⁷ hypertonic carbohydrate-electrolyte beverages (10% glucose, osmolality of 654 mosm.kg⁻¹) were as effective as hypotonic beverages (2% glucose and 188 mosm.kg⁻¹) and carbohydrate-free beverages (74 mosm.kg⁻¹) in restoring fluid balance when subjects consumed fluid *ad libitum*.

The palatability or taste of the fluid will dictate the amount consumed. Thirst mechanisms are inadequate to promote full fluid replacement when plain water is consumed.^{156,165} Several studies have shown that both adults⁵² and young children⁴⁷ consume more fluid when the beverage is flavored. In children, taste preferences dictate drinking volume, with sufficient consumption of fluid only occurring when a flavored beverage was consumed.⁴⁷ In adults, the addition of sodium to a rehydration beverage increases voluntary intake¹³⁹; however, high levels of sodium can make the drink unpalatable.¹⁶⁴ The addition of carbohydrate can offset the palatability issues with beverages that contain sodium. A study investigating the effect of palatability on voluntary intake of fluid after exercise found that participants drank 123% of their sweat losses with flavored water alone, 163% with flavored water and 25 mmol.L⁻¹ sodium, and 133% with flavored water and 50 mmol.L⁻¹ sodium.¹³⁹ Commercial sports drinks are designed to provide a balance between the efficacy of a higher sodium content and palatability through addition of carbohydrate. Other drinks, such as skim milk, can provide higher levels of electrolytes without issues of palatability.¹⁶² The athlete must weigh the benefits of consuming large volumes of a flavored beverage for adequate rehydration against the additional energy intake. Taste fatigue can also occur, particularly in endurance events when a single flavored drink is the only option available over an extensive period of time, or postexercise when a large volume needs to be consumed in a short time period. Including a variety of beverage flavors may encourage increased fluid consumption.

12.6 CONCLUSIONS AND FUTURE DIRECTIONS

Maintaining hydration status is a major challenge to the athlete, and particular efforts should be made to limit hypohydration and the potential impairment to physiological function and health. Fluid losses in excess of 2% of body mass have been shown to have a substantial impact on physical and cognitive performance in a variety of

climatic conditions. Despite knowledge of the importance of adequate rehydration strategies, many athletes fail to hydrate adequately before, during, and after exercise. Conversely, some athletes have a tendency to overdrink and risk developing hyponatremia. Therefore, all athletes should develop their own hydration strategy based on individual sweat losses in a variety of training and competition situations. Athletes should drink as much as practically possible but not more than their sweat loss. The palatability, temperature, and electrolyte content of the beverages they are consuming should be carefully considered, as this may play an important role in determining the amount of fluid consumed.

A wide variety of tools are available to assess hydration status, including body mass changes, bioelectrical impedance analysis and dilution techniques, hematological and urinary indices, and salivary parameters. A preferred tool should be noninvasive, economical with minimal consumables, technically simple to operate, stable, portable and robust, valid, precise, and not influenced by factors unrelated to hydration status. Given this, urinary indices (especially specific gravity and color) are generally the preferred method to assess hydration status, particularly in field settings. Upon waking urine samples are preferred as this avoids the potential confounding influence of acute food and fluid intake prior to urine sample collection. Hydration assessments can be undertaken at any time of the year but may have particular application during periods in which environmental conditions or training loads change. Assessments prior to competition may be best undertaken the day before competition, allowing hypohydrated athletes an opportunity to fully rehydrate prior to competition.

Although hydration assessment tools are becoming increasingly available, research supporting the interpretation of results is lacking. Cutoff points to identify a state of dehydration have been proposed, but little is known about the degree of total body water deficit associated with results from these field tools. Future research should investigate the association between changes in indirect indices of hydration status and total body water. This would afford a much more prescriptive approach to fluid intake following hydration assessments.

Ucol analyses reported in the literature have been based upon analysis by researchers rather than athletes. This approach lacks practicality because in practice, athletes are performing the analysis. Therefore, the relationship between Ucol (analyzed by athletes) and Uosm/Usg, and the strength of association between Ucol measured by a trained investigator in comparison to athletes should be investigated.

12.7 PRACTICAL RECOMMENDATIONS

- Assess individual hydration status and sweat rate to assist in the development of individualized fluid intake plans that are specific to different environmental conditions, exercise intensities, and sporting disciplines.
- Establish a routine of weighing in before and after training to determine sweat loss. This is particularly important when the individual is new to the training environment, there are high-intensity training sessions, or the envi-

ronmental conditions change. This allows prescription of individualized rehydration needs (that is, 150% of weight loss).

- Urine color is a simple, practical tool that requires minimal equipment and can be used for daily monitoring by the athlete.
- Biochemical indices of hydration status can be particularly valuable in providing an objective assessment of the athlete's ability to match fluid intake with fluid losses. However, given the cost, invasiveness, and difficulty with obtaining a blood sample, a measure of the specific gravity of an upon-waking urine sample can be a practical alternative.
- Collect and analyze sweat samples, and observe salt crusts on skin or clothing, and the taste of salt. Estimate the need for sodium replacement. Prescribe sodium replacement by adjusting food/fluid recommendations based on individual needs.
- Consider the needs of specific groups such as women, children and adolescents, and older individuals

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Section V

Clinical Assessment of Athletes

13 Clinical Assessment of Athletes

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13.1 INTRODUCTION

Athletic activities have been a part of competitive amateur and professional sports for years. Recently, the health benefits of exercise have resulted in an increasing number of previously sedentary persons participating in athletic activities. Older sedentary individuals are engaging in athletic activities to lose weight, reduce the risk of cardiovascular diseases, prevent diabetes, and correct progressive muscle wasting referred to as sarcopenia.

However, for the purpose of clinical assessment, it should be recognized that in competitive sports, persons tend to perform at the limits of their capabilities and relatively minor abnormalities in their cardiovascular system may result in fatal events. Similarly, older sedentary individuals have risk factors for coronary artery disease such as obesity, diabetes, and hypertension. In both competitive and recreational athletes, it is necessary to initially assess the person to determine whether he or she is in a good nutritional and physical status to engage in athletic activities. The potential competitive athlete often is underweight and on a restricted diet high in supplements with a view to having a very low body fat content and improving performance. In addition, he or she may have an undetected congenital heart disease. The elderly or sedentary recreational athlete may be, in contrast, overweight and on cyclical dieting followed by binge eating. In both groups, conditions likely to increase the risk of cardiac events and injuries need to be identified prior to initiating training.

After starting training, ongoing assessment is desirable in both types of athletes. In the competitive athlete there is considerable risk of malnutrition, especially in women. In the older athletes the objective is to promote weight reduction, document improvement in cardiovascular function, and observe a gain in muscle or lean body mass and muscle function. The assessment has two main sections: assessments of the nutritional status and the medical/physical status. Because cardiovascular health is especially a consideration for athletes, there should be additional emphasis on cardiovascular considerations.

13.2 NUTRITIONAL STATUS

The clinical assessment of nutritional status attempts to identify the initial nutritional state and the interplay of the factors influencing the progression or regression of nutritional abnormalities. Therefore, a clinical nutritional assessment is a dynamic process that is not limited to a single “snapshot” at the moment of measurement but provides a picture of current nutritional status and insight into the patient’s future status. The clinical assessment of nutritional status involves a focused history and physical examination in conjunction with selected laboratory tests aimed

at detecting specific nutrient deficiencies to identify patients who are at high risk for future nutritional abnormalities. A number of methods of nutritional assessment rely on measurements of height and weight and calculation of Body Mass Index^{1,2} (BMI): $BMI = [\text{height (meters)}]^2 / \text{weight (kg)}$, which is a way of normalizing weight by the height. In the West, in the Caucasian population, the mean normal BMI is 23, range (18–25) but in Oriental persons and in those from India the mean normal is 20 and several otherwise healthy persons have a BMI below the Western range. Hence, in this chapter the assessment is based on a “dimensionless” algorithm applicable to all ethnic groups, dependent on the direction of change and factors that are known to govern the nutritional status³ rather than simply a weight-to-height index.

13.2.1 HISTORY

The nutritional history should evaluate the following questions:

1. Has there been a recent unintentional change in body weight?
2. Is dietary intake adequate? The patient should be questioned about his or her habitual diet and any change in diet pattern. Has the number, size, and contents of meals changed? Are nutrient supplements being taken? A diary documenting food intake may be useful when the history is inconclusive. In this context, the diet of competitive athletes may be inadequate despite normal biochemical parameters.⁴
3. What are the reasons for the change in dietary intake? Has appetite changed? Is there a disturbance in taste, smell, or the ability to chew or swallow food? Has there been a change in mental status or increased depression? Has there been a change in the ability to prepare meals? Are there gastrointestinal symptoms, such as early satiety, postprandial pain, nausea, or vomiting? Is the patient taking medications that affect food intake?
4. Is there evidence of malabsorption? Is there any gastrointestinal disease? Has there been a change in bowel habits?
5. Are there symptoms of specific nutrient deficiencies including macronutrients, micronutrients, and water?

13.2.2 PHYSICAL EXAMINATION

The physical examination corroborates and adds to the findings obtained by history.

1. Anthropometric assessment. Current body weight should be compared with previously recorded weights, if available. If accurate weights are not available, assessment of the weight loss and whether it is continuing during the previous 2 weeks should be undertaken. A search for evidence demonstrating depletion of body fat and muscle masses should be made. A general loss of adipose tissue can be judged by clearly defined bony, muscular, and venous outlines, and loose skin folds. A fold of skin, pinched between the forefinger and thumb, can detect the adequacy of subcutaneous fat. The

presence of hollow cheeks, buttocks, and perianal area suggests body fat loss. An examination of the temporalis, deltoids, and quadriceps muscles should be made to search for muscle wasting.

2. Assessment of muscle function. Strength testing of individual muscle groups should be made to evaluate generalized and localized muscle weakness. In addition, a general evaluation of respiratory and cardiac muscle function should be made.
3. Fluid status appraisal. An evaluation for dehydration (hypotension, tachycardia, postural changes, mucosal xerosis, dry skin, and swollen tongue) and excess body fluid (edema, ascites) should be made.
4. Evaluation for specific nutrient deficiencies. Rapidly proliferating tissues, such as oral mucosa, hair, skin, and bone marrow are often more sensitive to nutrient deficiencies than are tissues that turn over more slowly.

13.3 MEDICAL STATUS

13.3.1 MEDICAL HISTORY

Clinical assessment should start with a careful history that should inquire about factors that are likely to influence the risks and benefits of exercise. In younger competitive athletes it is necessary to look for factors that are likely to be endangering health or risk death in the face of strenuous exercise.

13.3.1.1 Competitive Athletes

Cardiovascular system. There is concern of sudden cardiac death and cardiac events during athletic activities in individuals with known cardiac disease and those without. The two major groups to consider are screening of individuals who plan to participate in competitive athletic sports without a prior history of cardiac problems and how to advise and possibly restrict those individuals with known cardiac disease prior to undertaking athletics.

Sudden cardiac death (SCD) and cardiac events in athletes are rare events. Although the actual event rate is not known, it has been estimated to be in the order of 1:200,000 young people of high school age per year.⁵ More recent registry data in the United States over a 27-year period (1980–2006), has found that the rate is < 100 cardiovascular sudden deaths in young athletes per year.⁶ However, these events usually attract a lot of media attention as the event occurs in often young and presumed healthy individuals. The best approach to screen individuals, especially competitive athletes, is a source of major debate within the cardiovascular and athletic communities around the world. The purpose of preparticipation screening is to identify those individuals who may have clinically relevant preexisting conditions, and for the purpose of this chapter, those that are cardiac in nature. The screening process should provide potential competitive athletes with an assessment of their medical eligibility for competitive sports. Therefore, the purpose of the screening process is to reduce the risk inherent in competitive sports and thereby improve the safety of

competitive athletes in sport. It should be recognized, however, that it is not possible to achieve a zero-risk scenario for competitive athletes with preparticipation screening. However, if an abnormality is found, then the screening process is just the first step for the individual as further assessment and care will need to be sought through referral to a subspecialist. The subspecialist will need to determine if the individual is disqualified from competitive sport or if exercise restrictions need to be applied. This topic is beyond the scope of this chapter.

The most common cardiovascular causes of sudden death in competitive athletes in the United States who are less than 35 years old include hypertrophic cardiomyopathy (HCM) (36%), coronary artery anomalies (17%), indeterminate left ventricular hypertrophy (LVH) or possible HCM (8%), myocarditis (6%) and arrhythmogenic right ventricular dysplasia (4%).⁷ Sudden cardiac death in older athletes (older than 35–40 years old) is usually secondary to an arrhythmia in the setting of unsuspected atherosclerotic coronary artery disease. The estimated combined prevalence of cardiac disease in the general athlete populations is 0.3%.

The American Heart Association (AHA) updated its scientific statement on preparticipation screening of competitive athletes in 2007. The AHA recommendations include a twelve-element screening tool that breaks the assessment into medical history (including personal history and family history) and then physical examination.⁷ The European guidelines cite similar screening criteria based on history and physical examination.⁸ A positive response to one or more of the twelve items may be judged worrisome and indication enough to prompt a referral to a cardiologist at the discretion of the examiner. The Lausanne recommendations under the umbrella of the International Olympic Committee (IOC) medical commission (2004) outline a very extensive stepwise approach to the screening of athletes.⁹ If based on the preparticipation screening it is decided to refer the athlete to a cardiologist, then the athlete should not be permitted to practice or compete in events until final medical clearance has been given.

Medical history. Parental verification is recommended for middle school and high school athletes.

1. Personal History

- a. Exertional chest pain/discomfort
- b. Unexplained syncope/near-syncope (judged not to be vasovagal, and of particular concern when related to exertion)
- c. Excessive exertional and unexplained dyspnea/fatigue associated with exercise
- d. Prior recognition of a heart murmur
- e. Elevated systemic blood pressure

2. Family History

- a. Premature death (sudden and unexplained or otherwise) before age 50 due to heart disease in > 1 relative
- b. Disability from heart disease in a close relative < 50 years of age

- c. Specific knowledge of certain cardiac conditions in family members: hypertrophic or dilated cardiomyopathy, long QT syndrome (LQTS) or other ion channelopathies, Marfan's syndrome, or clinically important arrhythmias

Respiratory system. History of cigarette smoking and of asthma, particularly if intensified by exercise, should be obtained.

Nervous system. History of dizziness, especially induced by exercise or by lifting heavy objects should be ascertained prior to starting a program of aerobic or strength training exercise.

Musculoskeletal systems. History of previous injuries or operations on joints as well as the residual functional limitations should be evaluated. History of juvenile arthritis should be determined.

Metabolic conditions. Juvenile diabetes is a concern; if present, note the dose of insulin and control of blood glucose. Episodes of hypoglycemia should be avoided.¹⁰

13.3.1.2 Sedentary and Elderly Athletes

In addition to the history suggested above, those who are proposing to start recreational exercise and athletics should be questioned about symptoms suggestive of

1. Angina, dyspnea on exertion, strokes, and hypertension.
2. Diabetes and renal disease.
3. Body weight changes of either weight gain or unexpected weight loss should be obtained. The former increases the risk of exercise and the latter occurs with the onset of serious disease.
4. The existence of arthritis and limitations in mobility.
5. The previous exercise status and a drug history should be obtained. Drugs likely to influence exercise ability are
 - a. Beta-blockers, which cause fatigue and prevent exercise-induced tachycardia.
 - b. Insulin action will increase with exercise and may cause hypoglycemia.
 - c. Antihypertensive drugs may cause fall in blood pressure.

13.3.2 PHYSICAL EXAMINATION

Head and neck examined for enlarged nodes, thyroid enlargement. Eyes checked for central and peripheral vision and the cornea and conjunctiva examined for signs of vitamin A and riboflavin deficiency. Tongue and mouth examined for glossitis and stomatitis, which can suggest iron, folate, or vitamin B₁₂ deficiency.

Respiratory system for any abnormalities such as evidence of asthma, chronic bronchitis and emphysema.

Cardiovascular system

1. Heart murmur: Auscultation should be done in both supine and standing positions (or with valsalva maneuver), specifically to identify murmurs of dynamic left ventricular outflow tract obstruction.
2. Femoral pulses to exclude aortic coarctation.
3. Physical stigmata of Marfan's syndrome.
4. Brachial blood pressure in the sitting positions in both arms.

Abdomen for enlarged organs and for any masses or tender areas.

Central nervous system for cranial nerve function, general motor function, general sensory function, and coordination.

Musculoskeletal system for wasting, joint swelling, joint tenderness, and mobility both passive and active.

13.3.3 HEMATOLOGY

The hemoglobin level is essential to determine whether the person has sufficient oxygen-carrying capacity to undertake exercise. Particularly in women, marginal iron deficiency is extremely common because of menstrual blood losses. The hemoglobin tends to be at the lower limits of normal but the mean corpuscular volume (MCV) is reduced and the serum ferritin levels are markedly low. Since iron is an important part of a number of mitochondrial constituents such as cytochrome, deficiency results in reduced muscle performance and fatigue even when the hemoglobin is only slightly reduced. On the other hand, with vitamin B₁₂ and folate deficiency, the MCV is increased. An increase in the MCV should lead to measurements of blood B₁₂ and folate acid levels. An increase in white blood count and platelet levels are indicative of any inflammation.

13.3.4 BLOOD BIOCHEMISTRY

Blood glucose levels should be measured to exclude diabetes. Creatinine levels should be measured to exclude renal disease. Blood electrolyte levels, magnesium, calcium, and phosphorus should be checked. Abnormalities in any of these levels can alter muscle performance. Protein status can be assessed by the levels of prealbumin and blood urea nitrogen (BUN). In protein deficiency both these parameters are reduced. The serum albumin level is not indicative of nutritional status, and in adults hypoalbuminemia is an index of occult disease.¹¹ The levels of ferritin, vitamin B₁₂, and folate should be measured. Finally, total cholesterol, LDL cholesterol, and HDL cholesterol should be measured.

13.3.5 MICRONUTRIENT LEVELS

During athletic training, dietary intake may become imbalanced due to inadequate intake of foods providing micronutrients. Diets high in refined carbohydrates are often deficient in zinc, selenium, vitamins, and perhaps other minerals and vitamins such as magnesium. A recent study showed that there was significant vitamin B₆

deficiency in high-performance athletes.¹² Therefore if the diet of an athlete is unbalanced, micronutrient levels should be measured.

13.3.6 URINE ANALYSIS

Urine analysis should be done to look for renal disease. The presence of protein, casts, and red blood cells suggest kidney disease and should lead to further examination. Temporary abnormalities in the urine such as proteinuria, microscopic hematuria, and casts may be seen after prolonged vigorous exercise due to reduced renal blood flow and dehydration.

13.3.7 STOOL EXAMINATION

Stool examination for parasites such as hookworm may have to be performed in countries where this problem is endemic. Parasites cause iron deficiency, which in turn reduces exercise tolerance.

13.3.8 ELECTROCARDIOGRAPHIC AND ECHOCARDIOGRAPHIC EXAMINATION

13.3.8.1 Competitive Athletes

Preparticipation Screening Recommendations: It is recognized that preparticipation screening with only the history and physical examination does not have the sensitivity to guarantee detection of all cardiovascular abnormalities that could cause SCD. This could result in false-negative results.

Electrocardiogram (ECG): The 12-lead ECG is abnormal in > 90% of patients with HCM and can uncover ion channelopathies such as long QT syndrome and Brugada syndrome. However, the ECG is often abnormal in the hearts of trained athletes as a normal physiologic response.

The International Olympic Committee (IOC)⁹ and the European Society of Cardiology (ESC)⁸ currently advocate that all young competitive athletes be screened routinely with a 12-lead ECG in addition to the history and physical examination. The rationale for the routine use of the ECG for preparticipation screening is based on the Italian experience. Italy has a 25-year experience with a state-subsidized national program in which all individuals participating in sport between the ages of 12 and 35 are mandated to be screened with a history, physical examination, and ECG by an accredited sports medicine physician.¹³ This program has been successful for detecting cardiac disease that could result in SCD and has resulted in the disqualification of athletes. This action coincided with an almost 90% reduction in the annual incidence of sudden cardiovascular deaths in competitive athletes. Currently the AHA 2007 guidelines do not make this recommendation as the routine use of ECGs for preparticipation screening would not fit in the current U.S. model of athletics. However, for the older athlete, the routine use of the ECG is recommended by the AHA.

Exercise Stress Testing: Exercise stress testing can be used to detect catecholaminergic polymorphic ventricular tachycardia and for the detection of coronary artery disease. Stress testing for the detection of congenital anomalies of the coronary arteries can often give false-negative results. For the detection of atherosclerotic coronary artery disease (CAD), the exercise stress test has low specificity in the low-risk athlete. The routine use of exercise stress testing in healthy asymptomatic athletes with a low-risk profile is not recommended as there are problems with poor positive predictive accuracy and false-positive tests that can have negative implications and result in further unnecessary testing. However, the older competitive athlete should have focused screening for previously undiagnosed atherosclerotic coronary artery disease. If, based on the preparticipation screening tool (as outlined above), the older athlete has no evidence of coronary artery disease but he or she does have a moderate- to high-risk cardiovascular profile for CAD and wishes to participate in vigorous competitive situations, he or she should undergo exercise stress testing.¹⁴ The risk profile would include men > 40–45, women > 50–55 years of age (or postmenopausal), with one or more independent risk factors including the following: dyslipidemia, systemic hypertension, smoker, diabetes mellitus, family history of SCD or myocardial infarction in a first-degree relative < 60 years old. If there are symptoms suggestive of CAD based on the history or if the athlete is > 65 years old regardless of his or her risk profile, and even when asymptomatic, an exercise stress test should be performed. However, a more recent study has found that the routine use of an exercise ECG in people seeking to obtain clinical eligibility for competitive sports can identify those individuals with cardiac abnormalities who would have otherwise passed preparticipation screening based on history, physical exam, and resting ECG.¹⁵ This study identified that participants over the age of 30 had a significantly increased risk of having cardiac abnormalities on the exercise ECG, thus resulting in disqualification from competition. However, follow-up studies are needed to show if this screening process would reduce the incidence of cardiac events in athletes.

Echocardiogram: This test is without any risk and can be diagnostic for several conditions that cause SCD in athletes. Cardiac conditions such as HCM, aortic stenosis, mitral valve prolapse, aortic dilatation, bicuspid aortic valve, left ventricular dysfunction, and on occasion anomalous coronary arteries can be diagnosed with echocardiogram. However, the yield of routine echocardiography in all competitive athletes will be low.

13.3.8.2 Sedentary and Elderly Athletes

The American Heart Association recommends exercise testing with an electrocardiogram before the start of a vigorous exercise program for all individuals older than 40 years, even if they are asymptomatic and free of cardiac risk factors.² However, a study of more than 3,000 asymptomatic men aged 35 to 59 years, with increased risk of coronary artery disease, casts doubt on the value of this recommendation. Each subject had an exercise test on entry and annually for 7.4 years.

Exercise proved safe in this group, with approximately 2% experiencing exercise-related cardiac events. Only 11 of the 62 men who experienced such events had abnormal exercise tests on entry, a sensitivity of only 18%. The cumulative sensitivity of annual tests was also low, at 24%.¹⁶ However, exercise testing can be useful for detecting exercise-induced arrhythmias and for determining the maximal heart rate for the exercise prescription.

Echocardiography can be used to study the thickness of the ventricular wall, ventricular diameter, and valve function. In addition, any injury to the myocardium can be assessed by changes in the motion of the ventricular wall. Patients who have any history suggestive of coronary artery disease, those with significant risk factors such as hypertension, hypercholesterolemia, family history of myocardial infarction below the age of 50, diabetes, and obesity would be wise to have an echocardiographic assessment by a cardiologist prior to embarking on an exercise program for the first time after the age of 40.

13.3.9 BONE MASS

13.3.9.1 Competitive Athletes

In the competitive athlete, especially in women who undertake intense aerobic physical training, bone loss and even osteoporosis has been recognized.¹⁷ The so-called triad of amenorrhea, eating disorder, and bone loss may occur. The reason is a combination of reduced estrogen levels, reduced body weight and fat, and an imbalanced diet.¹⁸ In these women it is important to document the initial BMI and changes with training. Marked reduction in BMI and the development of amenorrhea or disordered menstruation should lead to checking the bone mineral density (BMD) by the use of dual energy x-ray absorptiometry (DEXA). If the BMD in an individual is more than 2.5 standard deviations (SD) below the mean for a young matched (for age and sex) population, the diagnosis is osteoporosis. If the BMD is below 1–2.5 SD of the mean for a young matched population, then the diagnosis is osteopenia.

13.3.9.2 Sedentary and Elderly Athletes

In these persons there may be existing osteopenia or osteoporosis on entry to the training program. In these subjects exercise has a beneficial effect and increases BMD.¹⁹ During exercise training, in persons with osteopenia or osteoporosis, a yearly record of changes in BMD is essential. Recording reduced BMD in the hip is important as it is especially resistant to treatment. In this situation a combination of jumping, stepping, marching, and side-stepping exercise is one of the few techniques capable of increasing BMD of the hip.²⁰

13.3.10 MUSCLE FUNCTION

13.3.10.1 Strength

Clinical strength in nutritional studies has been assessed in the upper limb with a hand-held dynamometer and shown to be improved by nutritional supplementation.²¹ Hand-grip strength is simple and easy to do and is well correlated with skeletal

muscle strength. Maximum grip strength and mean value of a 10-second sustained grip are completed with the dominant hand according to the method outlined in Sunnerhagen et al.²² Briefly, the patient is seated in a chair without arm rests and with the lowest rib level with the edge of the table. The shoulder is adducted and the elbow flexed to 90° to 100°. The other arm rests on a table. The palm and fingers are clasped around the handle and the force exerted against the transducer in the handle is recorded.

Another way of measuring the effect of nutrition on the strength of different muscle groups is the maximal weight that can be lifted fully one time only (1RM).²³ This measurement can be done for different muscle groups of the upper and lower limbs. Other methods to measure strength are the vertical jump and isokinetic extension at different rates that correlate with performance.²⁴

13.3.10.2 Endurance

In a nutritional study of anorexic patients,²⁵ the effect of feeding on endurance was tested by the an ergometric bicycle protocol (3-minute steps of 30 W) before and after 8, 30, and 45 days of refeeding. Before refeeding, the workload reached during the exercise was 49% lower in anorexia nervosa (AN) patients than in control subjects ($P < 0.01$) and was correlated with body weight, fat-free mass, and leg muscle circumference ($P < 0.002$). The performance improved dramatically during refeeding ($P < 0.03$), reaching normal values after 45 days of refeeding, despite fat-free mass and leg muscle circumference values that were still 20% lower in AN patients than in control subjects ($P < 0.01$).

13.3.10.3 Peak Performance and Duration

Peak performance can be tested by a modified Wingate protocol. In a study of nutritional supplements,²⁶ volunteers were subjected to a protocol in which they were tested for the duration of maximal performance at 110% of VO_2max . The subjects were tested for their VO_2max and then placed on a bicycle ergometer. The protocol consisted of a 3-minute warm-up, 1 minute of 40% VO_2max followed by 1 minute of 110% of VO_2max for four trial times. Then the subject bicycled until exhaustion at 110% VO_2max . In this study the supplement improved peak performance above that seen with placebo.

13.4 CLINICAL CONDITIONS ASSOCIATED WITH ATHLETIC ACTIVITIES

13.4.1 ELIGIBILITY RECOMMENDATIONS FOR COMPETITIVE ATHLETES

Current practices differ between high schools, colleges, and Olympic athletes versus professional athletes in the United States. Professional athletes often have more rigorous assessments compared to athletes in other venues, and in addition to the history and physical examination, often undergo noninvasive testing such as ECG, echocardiogram, and exercise stress testing. However, the AHA in their 2007 recommendations does not believe that it is either prudent or practical to recommend the routine use of ECG or echocardiogram in the context of mass universal screening.

However, when considering the individual athlete, noninvasive testing such as ECG and echocardiogram can enhance the diagnostic power above the standard history and physical examination without risk to the patient. Therefore, the use of such non-invasive testing should be considered. At a minimum, preparticipation screening should include the twelve elements of the history and physical as outlined in [Section 13.3](#), and the examination should be performed by a qualified examiner in an environment conducive to auscultation.

13.4.2 ELIGIBILITY RECOMMENDATIONS FOR COMPETITIVE ATHLETES WITH CARDIOVASCULAR ABNORMALITIES

For individuals with known prior cardiac disease or for those athletes who are found to have underlying cardiac abnormalities based on preparticipation screening, specialized evaluation by a cardiologist is recommended. The patient will need to have a complete cardiovascular evaluation to thoroughly assess the underlying cardiac abnormality, assess the stability of the cardiac condition, and be risk stratified for future cardiac events. A publication of the 36th Bethesda Conference is a useful guide for physicians and outlines recommendations based on the specific underlying cardiac diagnosis for the eligibility for athletics, the recommended intensity of the sport, and patients who should be disqualified from competitive sports.²⁷

13.4.3 DEHYDRATION AND ELECTROLYTE DEFICIENCIES

Prolonged exercise is associated with the loss of sweat and dehydration. Dehydration can cause reduction in reaction times and vigilance.²⁸ Dehydration can be avoided by ingestion of fluids during endurance exercise. Hyponatremia (serum sodium < 135 mmol/L) may occur due to excessive fluid intake rather than sodium loss. In a recent prospective study, dehydration accounted for 26% and hyponatremia for 9% of individuals who sought medical care during endurance exercise. Hyponatremia was the most common reason for hospital admission. There was an inverse relationship between postrace sodium concentrations and percentage change in body weight, supporting the suggestion that fluid overload is the cause of hyponatremia.²⁹

Plasma potassium rises in healthy subjects by only 0.5 mmol/L when exercising at 40–50% of $\text{VO}_{2\text{max}}$. Hyperkalemia occurs during strenuous exercise and is especially likely to occur in patients with angina or hypertension on beta-blockers such as propranolol.³⁰ Acidosis can occur with exercise³¹ but feeding alkalinizing agents does not improve performance.³²

13.4.4 ASTHMA

Exercise-induced asthma is a well-recognized clinical condition. It has been shown to occur to a greater degree in athletes who pursue strength (3.5 times more common than the general population) and endurance exercise (2.2 times more common). It occurs more frequently in women and in those who train more than 20 hours in the week.³³

13.4.5 ARRHYTHMIA

Cardiac events during exercise occur in about 2% of individuals over 40 years of age.² Even in those who are 75 years of age, exercise-induced arrhythmia occurred in ~24% of males and only ~7% of females but had no lasting effects.³⁴ However, asymptomatic persons who develop ventricular premature depolarizations had 2.6 times the risk of coronary events on followup.³⁵ On the other hand, exercise training of patients who have had myocardial infarction was shown to be of significant clinical benefit.³⁶

13.4.6 RENAL FAILURE

Frank renal failure may occur with severe dehydration during endurance exercise. It may rarely occur due to muscle breakdown called rhabdomyolysis.³⁷ On the other hand, exercise improved physical functioning in patients on dialysis.³⁸

13.4.7 GASTROINTESTINAL DISTURBANCES

Gastrointestinal disturbance is common among athletes during competition. These symptoms include nausea, vomiting, belching, heartburn, chest pain, bloating, abdominal cramps, urge to defecate, frequent defecation, and diarrhea.³⁹ The incidence of bloating, abdominal cramps, and diarrhea was higher with running. In contrast, all the above symptoms were equally likely to occur during bicycling.²⁰ Measurement of occult blood in the stools showed that during endurance exercise there was increased loss of blood in the stool.⁴⁰ The magnitude of the blood loss was small and did not alter ferritin levels. The cause of blood loss is uncertain but a possible explanation is that during intense exercise, especially during running, blood is diverted away from the bowel to the muscles, resulting in ischemia of the intestine.⁴¹ Athletes often take nonsteroidal analgesic drugs (NSAIDS), and the intake of these drugs has been shown to increase intestinal permeability and have more adverse gastrointestinal symptoms.⁴² Therefore gastrointestinal symptoms are partly due to exercise and partly due to drugs taken to alleviate pain.

13.4.8 ANEMIA

Plasma volume expansion occurs in athletes and contributes to the commonly occurring normocytic and normochromic anemia.⁴³ This “anemia” did not reduce performance. In football players a study showed that individuals with a lower hematocrit paradoxically had better aerobic capacity.⁴⁴ Iron deficiency does not occur simply due to exercise *per se*.⁴⁵ Anemia in athletes should not be dismissed as being due to exercise without proper investigation. Anemic athletes, especially older persons, could have an underlying serious condition such as colon cancer. The ingestion of NSAIDS can be associated with peptic ulceration and blood loss. Anemia could also be due to menstrual losses in women⁴⁶ when combined with a diet low in iron. In women, continuous physical exertion as in military combat training, which would be

equivalent to vigorous athletic training, reduces ferritin levels; iron supplementation improved vigor and running times.⁴⁷

13.4.9 IMMUNE DEFICIENCY

A heavy schedule of training has been shown to be associated with a depression of immunity.³³ The causes for immune depression are multifactorial. Imbalanced diet can influence immunity. During intense exercise the demand for carbohydrate by muscle is high and competes with the needs of macrophages and for purine and pyrimidine synthesis. A high carbohydrate intake may lead to reduced intake of proteins and lipids, which are important in the maintenance of immune function.⁴⁸ Intense training is also associated with reduced immunity due to excessive weight loss and reduced plasma glutamine levels.⁴⁹ Since glutamine is an essential nutrient for lymphocytes, the reduction of glutamine can interfere with lymphocyte function.

13.5 SUMMARY

Clinical assessment of the athlete is a comprehensive process that involves an evaluation of the current nutritional status and the possible change in nutritional status with athletic activity. In addition it is desirable to assess the medical condition of the person embarking on athletic activities. While assessing the medical condition of the potential athlete, it is necessary to differentiate the medical status required for the competitive athlete from those who are going to undertake recreational activities. Athletic activities can cause complications that the clinician should recognize and prevent or treat.

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