Nutrition and the Female Athlete

From Research to Practice

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Edited by

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
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CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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Version Date: 20121210

International Standard Book Number-13: 978-1-4398-4939-2 (eBook - PDF)

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Preface

Research examining the effects of nutrition on sports performance (i.e., sport nutrition) has exploded over the last 25 years. The interest in and proliferation of sport nutrition research has led to the creation of two scientific journals, numerous textbooks, and countless Web sites devoted specifically to this topic. Sports dietitians, coaches, and trainers are basing nutrition recommendations to their female athletes on the existing sport nutrition research. Similarly, the existing sport nutrition research is guiding and marketing the manufacturing of sport nutrition products to female athletes. All the while, a key limitation of much of the existing sport nutrition research is being overlooked; with a few exceptions, the majority of studies have employed only male subjects. The results of these studies are then generalized to females with no scientific support for the validity or accuracy of such a generalization. This oversight is not exclusive to sports nutrition research. One need only review the history of sports in general to appreciate the relative dominance by men and limited inclusion of women in all facets of athletics.

In the United States, relatively few women competed in sports until the late 19th and early 20th centuries, when social changes in North America favored increased female participation in society as equals with men (Oglesby 1978). Although women were technically permitted to participate in sports, relatively few did due to the social stigma as well as concerns regarding the effects of strenuous exercise on a woman's physical "constitution" (in particular, her reproductive health) (Oglesby 1978). Two World Wars and a couple of key social "movements" (i.e., the civil rights and women's movements) brought more women onto the athletic playing fields. However, it was not until 1972 when the U.S. Congress passed Title IX of the Educational Movements that the door to women's participation in sports was truly opened (Bell 2007). Subsequent to Title IX, women and girls have become much more involved in sports. College women's athletic participation has increased from 15% in 1972 to 43% in 2001. High school girl's athletic participation increased from 295,000 in 1971 to 2.8 million in 2002–2003, an increase of over 840% (Carpenter and Acosta 2005).

With an increase in women's sports participation has come a greater recognition of and appreciation for the biomechanical and physiological differences between men and women. Over the last 30 years, sports equipment and clothing manufacturers have become more interested in developing and marketing products specifically for women. I have witnessed this "evolution" personally. In the late 1970s, when I first took up distance running, there were no "women's running shoes"; I had to make do with the smallest-sized men's shoe I could find. Similarly, if I wanted nylon running shorts I had to search the boy's or young men's department. Today, every major running shoe manufacturer has not one but several shoes designed specifically for women, and there are numerous clothing lines that cater specifically to women's exercise wear.

Thanks in large part to the innovative studies conducted in the early 1990s by Mark Tarnopolsky and others at McMaster University (Ontario, Canada), researchers

are also starting to appreciate that the physiological differences between men and women may translate into different responses and adaptations to nutritional manipulations and, thus, different recommendations in terms of sport nutrition practices.

Therein lies the premise of this book.

It is now well established that females are metabolically unique from their male counterparts; thus, their nutritional requirements for optimal training and athletic performance are likely also unique.

Chapter 1 sets the stage for the discussion of gender-specific nutrition recommendations by highlighting recent research indicating that substrate utilization during exercise differs significantly between men and women. Written by the pioneer in this particular topic area, Mark Tarnopolsky, and one of his recent PhD students, Amy Maher, provide a number of possible explanations for the gender differences in substrate utilization as well as implications for gender-specific nutritional recommendations.

Louise Burke and Christine Dziedzic tackle the topic of carbohydrate needs of female athletes in Chapter 2. More specifically, the validity of generalizing the current guidelines for carbohydrate replacement before, during, and after exercise to female athletes is examined. Examples of ways in which female athletes can address their carbohydrate intake goals in the context of other nutritional needs and dietary concerns are also provided.

In Chapter 3, Nancy Rodriguez addresses the importance of adequate dietary protein in the diets of female athletes by reviewing the myriad of structural and functional roles that protein plays within the athlete's body. The effects of inadequate protein intake, particularly in combination with inadequate energy intake, on the health and performance of the female athlete are discussed and are used to guide recommendations for dietary protein intake.

Proper hydration is as important as carbohydrate and protein intake in terms of optimizing performance and the overall health of the female athlete. Dehydration negatively impacts performance as water increases body core temperature, heart rate, glycogen utilization, and perceived exertion. Nina Stachenfeld has spent a significant portion of her research career investigating the effects of reproductive hormones on the fluid and temperature regulatory systems in women. In Chapter 4, she and her former postdoctoral associate, Megan Wenner, examine sex differences in thermoregulation and fluid balance in order to determine whether female-specific fluid recommendations are necessary.

Although they do not provide energy or support hydration, there is no question that micronutrients (i.e., vitamins and minerals) play a critical role in supporting training, competition, and the overall health of the female athlete. Nonetheless, research indicates that female athletes often have suboptimal micronutrient intakes that place them at risk for deficiency. Nutrients that seem to be of particular concern for female athletes are discussed in Chapters 5 through 7. In Chapter 5, Pamela Hinton highlights the importance of iron and zinc for athletic performance and provides suggestions for helping female athletes meet their iron and zinc requirements. Bone nutrients are covered in Chapter 6 by Kristine Spence. In Chapter 7, Kathleen Woolf, Dara LoBuono, and Melinda Manore provide a comprehensive review of the exercise-related functions, food sources, and recommended intakes for each of the B vitamins.

The final four chapters of the book (i.e., Chapters 8 through 11) are devoted to a discussion of a set of three distinct yet often interrelated disorders including low energy availability, menstrual dysfunction, and poor bone health that have come to be known as the female athlete triad (Triad). Katherine Beals examines the concept of energy availability and summarizes the existing research regarding the etiology, prevalence, and consequences of low energy availability among female athletes. The foremost expert in endocrinology and the female athlete, Anne Loucks, provides a comprehensive review of the research examining menstrual dysfunction among female athletes with an emphasis on its prevalence, causes, consequences, and treatment options. Finally, Michelle Barrack addresses the third and final component of the Triad, bone health. Her chapter highlights genetic and lifestyle characteristics, including sport-specific factors that affect bone health, and provides behavioral recommendations female athletes can employ to optimize bone health and reduce their risk of musculoskeletal injuries.

REFERENCES

Bell, R. C. A history of women in sport prior to Title IX. *The Sport Journal*. 2007. Volume 10. http//www.thesportjournal.org/article/history-women-sport-prior-title-ix. Accessed May 1, 2012.

Carpenter, L. J., and R. V. Acosta. 2005. *Title IX*. Champaign, IL: Human Kinetics.

Oglesby, C. A. 1978. *Women and Sport: From Myth to Reality*. Philadelphia, PA: Lea & Febiger.

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Dr. Beals holds a PhD in exercise science and physical education from Arizona State University, is a registered dietitian, a fellow of the American College of Sports Medicine, and a Certified Specialist in Sports Dietetics. She has published more than a dozen articles and several book chapters on disordered eating and the female athlete triad. In addition, she has published two books on disordered eating including *Disordered Eating among Athletes: A Comprehensive Guide for Health Professionals* (Human Kinetics, 2004) and *The Hidden Faces of Eating Disorders and Body Image* (Human Kinetics, 2009).

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1 Substrate Utilization in Female Athletes
Implications for Fuel Selection and Macronutrient Requirements

Amy C. Maher and Mark A. Tarnopolsky

CONTENTS

INTRODUCTION

Until recently, it was assumed that men and women responded similarly to the metabolic stress of exercise; however, accumulating evidence supports that sex (gender) influences fuel metabolism during exercise. Specifically, controlled studies accounting for menstrual cycle phase, diet, habitual training, and aerobic capacity have consistently shown that women have higher relative fat oxidation and lower protein and carbohydrate (CHO) oxidation during submaximal intensity exercise as compared to men (Tarnopolsky et al. 1990; Phillips et al. 1993; Tarnopolsky et al. 1995; Friedlander et al. 1998; Horton et al. 1998; Davis et al.

2000; Carter et al. 2001a; Lamont et al. 2001b; Ruby et al. 2002; Devries et al. 2005). Sex differences in metabolism are likely genetically regulated either by predetermined expression of genes or by the regulation of gene expression through cell signaling mechanisms, likely mediated through sex hormones (estrogen, progesterone, and testosterone) (Wolfe et al. 2000; Ferrando et al. 2002; Fu et al. 2009; Maher et al. 2009). Despite the differences in substrate utilization during exercise, the adaptations in aerobic capacity to endurance exercise training appear to be similar between men and women (Friedlander et al. 1998; McKenzie et al. 2000; Skinner et al. 2001).

Research examining the impact that metabolic differences due to sex, menstrual cycle, and age have on nutritional recommendations in recreational and top sport female athletes is still in its infancy. Thus, at this time, we can only speculate on how sex differences in substrate utilization may affect nutritional recommendations for the physically active woman. However, recent advances in modern techniques such as proteomics and gene expression array analysis are proving useful in helping us understand the molecular basis for differences in these areas. This chapter will focus on describing sex differences in macronutrient fuel selection, the possible mechanisms for these differences, and implications that these differences may have with respect to nutritional recommendations to optimize performance for the female athlete.

SEX DIFFERENCES IN SUBSTRATE UTILIZATION DURING ENDURANCE EXERCISE

For years it was assumed that substrate utilization during exercise was similar between men and women. This assumption was based largely on a study conducted by David Costill and colleagues (1976) in which they compared trained women with men (track athletes) and also untrained women with untrained men. Their results suggested that when compared based on training history, men and women had similar VO_{2max} (ml/kg × min^{-1}), enzyme activity, and muscle fiber types (Costill et al. 1976). Consequently, much of the research regarding energy metabolism and fuel utilization during exercise has been conducted predominately on men as there was no reason to believe that the generalization of results did not apply to women. Indeed, it was not until the last decade that researchers began to seriously consider the probability of sex differences in exercise substrate metabolism.

In 1990 Tarnopolsky and colleagues compared substrate utilization in women and men during an acute treadmill run at 65% of VO_{2max} (Tarnopolsky et al. 1990). The women and men were matched for training history and consumed a controlled isocaloric diet for 3 days prior to testing (55% CHO, 30% fat, 15% protein). The women had a significantly lower respiratory exchange ratio (RER)*, 25% lower muscle glycogen utilization, and 30% lower urea nitrogen excretion (Tarnopolsky et al. 1990).

* RER is the ratio of the volume of carbon dioxide eliminated from the lungs to the volume of oxygen taken into the lungs per minute and gives an estimation of the ratio of fuel being metabolized (e.g., pure fat has an RER of 0.7 while pure CHO has an RER of 1.0).

These results were pivotal in demonstrating that during submaximal, long-duration exercise, women utilize more fat and less CHO and protein compared with men.

Most cross-sectional studies have found that whole-body oxidation rates are different for women as compared with men during endurance exercise at submaximal exercise intensities (Froberg and Pedersen 1984; Blatchford et al. 1985; Phillips et al. 1993; Tarnopolsky et al. 1995, 1997; Friedlander et al. 1998; Horton et al. 1998; Goedecke et al. 2000; McKenzie et al. 2000; Carter et al. 2001a; Lamont et al. 2001a; Melanson et al. 2002; Roepstorff et al. 2002; Steffensen et al. 2002; Lamont et al. 2003; Zehnder et al. 2005; Devries et al. 2006; Horton et al. 2006; Roepstorff et al. 2006; Wallis et al. 2006; Pillard et al. 2007; Kang et al. 2009; Maher et al. 2010b). Specifically, women demonstrate a relatively greater fat oxidation and concomitantly lower CHO and protein oxidation compared to men at the same relative exercise intensity ranging from 50 to 70% VO_{2max}. This has been demonstrated in studies employing cycling as well as treadmill running as the mode of exercise for duration of 60 to 120 minutes. These sex differences in substrate oxidation can be observed in both trained (exercise three or more times a week) and untrained (do not exercise) men versus women (reviewed in Tarnopolsky 2008).

It should be noted that not all studies have found gender differences in whole-body substrate utilization (Costill et al. 1979; Davis et al. 2000; Romijn et al. 2000; Mittendorfer et al. 2002; Riddell et al. 2003; M'Kaouar et al. 2004), partly due to methodological differences (which will be described in detail in the next section). Nonetheless, when whole-body RER values from all gender comparative studies were combined into a "meta-analysis" (even those not showing an effect), the specific sex differences in substrate utilization were maintained (Tarnopolsky 2008). Specifically, the results of the meta-analysis supported the relatively greater fat oxidation of women compared to men (~62% versus 43%, respectively) and relatively higher CHO and protein oxidation rates for men compared to women (52% versus 36% and 5% versus 2%, respectively) (Phillips et al. 1993; Tarnopolsky 2000; Lamont et al. 2001a, 2005).

METHODOLOGICAL CONSIDERATIONS FOR MEASURING SUBSTRATE UTILIZATION IN FEMALE ATHLETES

Whole-body substrate metabolism has generally been evaluated during endurance exercise using indirect calorimetry, whereas RER is used for the estimation of whole-body fat and CHO utilization. There are several factors that may alter substrate oxidation rates during exercise that must be controlled for when conducting studies designed to evaluate the effects of gender differences in fuel selection and substrate utilization. Specifically, the subject's size (weight), training status and history, and habitual and pre-exercise dietary intake status. On average, women have a higher percentage of body fat (~5 to 10%) and lower muscle mass compared with similar age- and activity-matched men (Tarnopolsky et al. 1990, 2000; Carter et al. 2001a; Tarnopolsky, Zawada et al. 2001). Therefore, it is important to express the main indicator of fitness (VO_{2max}) relative to lean body mass (ml O_2/kg LBM/min), as comparisons based on absolute VO_{2max} would lead to the selection of females who are heavier than the males. Similarly, exercise training has multiple consequences on

physiological and metabolic function, which ultimately alters VO_{2max}, which is why subjects should also be matched based on training history (Cureton and Sparling 1980). To overcome the issue of training history, a longitudinal approach can be taken in research such that untrained people are placed on a set exercise program to ensure equality of training (Devries et al. 2008). This matching approach takes into account both environmental (training state) factors and genetic (VO_{2max} potential) factors that contribute to VO_{2max} and expresses them relative to the mass of metabolically active tissues (Tarnopolsky and Saris 2001).

Habitual dietary intake should be compared between men and women in the same study, as high-CHO-low-fat diets lead to a greater reliance on CHO metabolism and low-CHO-high-fat diets lead to a greater reliance on fat metabolism (Spriet and Peters 1998), which could ultimately skew results if one sex prefers a diet slightly different than the other. It is noteworthy that most sex-based studies comparing habitual diet records from men and women show no significant difference in the ratio of CHO:fats:protein (Roepstorff et al. 2002; Timmons et al. 2005; Devries et al. 2006; Tarnopolsky et al. 2007; Fu et al. 2009; Maher et al. 2010b). Dietary intake prior to testing should also be controlled by administering an isocaloric (kcal/kg) meal at the same time as pre-exercise in both men and women for the same reasons listed above.

Because the relative ratio of female sex hormones (i.e., estrogen:progesterone) can influence substrate utilization, women should all be in the same phase of their menstrual cycle, and menstrual irregularities such as oligoamenorrhea and amenorrhea must be controlled or accounted for (Nicklas et al. 1989; Campbell, Angus et al. 2001; Devries et al. 2006). Studies should also consider oral contraceptive use, as oral contraceptives have a slight effect on substrate selection during endurance exercise, with a higher glycerol rate of appearance (lipolysis) (Devries et al. 2006).

Sex comparison studies should also test men and women during the same experimental time period (and not with historical data) to control for variations in metabolic assessment equipment (i.e., metabolic carts) and equipment calibrations (i.e., calibration of gas supply), as well as the research staff responsible for subject testing. Subjects must be in a steady state of exercise intensity and exercising below the anaerobic threshold (<75% of VO_{2max} in trained athletes and <65% for untrained individuals) as sex comparisons of substrate oxidation rates exceeding the lactate threshold do not yield accurate or valid results due to hyperventilation (inaccurate RER), rapid fatigue, and inability to sustain the work intensity (Tate and Holtz 1998). Taken together, it is important to consider the aforementioned factors to reduce variance and the potential to produce false conclusions regarding sex differences. The following sections describe sex differences in CHO and fat oxidation during endurance exercise and the role that estrogen plays in modulating substrate use.

SEX DIFFERENCES IN CARBOHYDRATE METABOLISM DURING EXERCISE

Overview of Carbohydrate Oxidation

The primary substrate sources for sustaining muscle contraction during submaximal endurance exercise are CHO and fat, with protein contributing only a small amount

of energy (2 to 5%) under normal circumstances (Lamont 2005). CHOs are stored in the muscle (1 to 2% of total muscle mass) and liver (<8% total mass, 100 to 120 g) in the form of glycogen. Glycogen provides a rapid source of energy to the cells during exercise, particularly at intensities greater than 50% of VO_{2max} (Powers and Howley 1996). Glycogen stores are limited and can be depleted within a couple of hours of prolonged exercise, depending on intensity and fiber type. The greater the exercise intensity and the more fast twitch fiber recruitment, the more rapidly glycogen will be depleted (Gollnick et al. 1974). Endurance performance can be altered by changing habitual dietary CHO intake, increasing CHO intake prior to an event (CHO loading), consuming CHOs during an event, and consuming CHOs immediately post-exercise, ultimately changing substrate utilization. (For more on this topic see Chapter 2.)

Sex Differences in Carbohydrate (CHO) Oxidation

As previously mentioned, the majority of cross-sectional studies have found that whole-body CHO oxidation rates are lower for women compared to men during endurance exercise (running, walking, and cycling) at submaximal exercise intensities (45 to 70% VO_{2max}), for durations ranging from 60 to 120 minutes (Tarnopolsky 2008). Moreover, these differences persist after 2 to 3 months of monitored exercise training (Friedlander et al. 1998; Horton et al. 1998; McKenzie et al. 2000; Carter et al. 2001a), further supporting the notion that the sex-based differences in substrate selection are attributable to sex, and not matching issues related to unequal training status.

The mechanism for the reduction in CHO oxidation in women is unclear. There does not seem to be a sex difference in basal muscle glycogen content in trained or untrained women compared with men (McKenzie et al. 2000; Tarnopolsky, Zawada et al. 2001). This suggests that the difference in CHO utilization between men and women likely involves an attenuation of hepatic and muscle glycogen utilization in women. In support of this notion, several studies have shown that the rate of glucose appearance (Ra) and disappearance (Rd) was lower in women compared with men (Roepstorff et al. 2002; Devries et al. 2006; Horton et al. 2006). In one particular study, the A-V (arteriovenous) balance method was used to examine substrate use across the leg during 90 min of bicycle exercise at 58% of VO_{2max} in seven endurance-trained men and women (Roepstorff et al. 2002). The results indicated that the glucose rate of appearance into the blood from the liver was lower for women (Roepstorff et al. 2002), suggesting that at least some of the sparing of CHO oxidation in women must involve the liver.

Sex differences in CHO utilization may be due to upstream biological factors such as glucagon, epinephrine, and sex hormones. Glucagon is a hormone secreted by the pancreas that stimulates liver and muscle glycogenolysis and release of glucose from the liver to maintain blood glucose levels. Horton et al. (2006) and Tarnopolsky et al. (1990) reported that men had a greater reduction in blood glucagon levels during exercise compared with women (Tarnopolsky et al. 1990; Horton et al. 2006). If the liver takes up glucagon (binds receptors or is internalized into the cells) in order to promote glycogenolysis, then these results could suggest that glucagon may partially

account for the observed sex difference in glucose rate of appearance. During endurance exercise women have been shown to have a lesser increase in plasma epinephrine concentration as compared with men (Brooks et al. 1990; Tarnopolsky et al. 1990; Ruby et al. 1997; Horton et al. 2006). Epinephrine stimulates glycogenolysis in the liver and muscle, and stimulates glycolysis in muscle. Interestingly, adipocytes from women show higher sensitivity to epinephrine as compared with men (Jensen et al. 1996; Monjo et al. 2003; Ramis et al. 2006), implying that there are sex differences in adrenergic receptor density or post-receptor regulation, at least within the adipocytes, that may account for sex difference in substrate utilization.

Sex hormones (specifically estrogen) show the strongest *association* with the observed sex-based differences in substrate metabolism. Women in both the follicular and luteal phases of their menstrual cycles had a lower glucose Ra, glucose Rd, and metabolic clearance rates as compared with men during 90 min of cycling at 65% VO_{2max} (Devries et al. 2006). Moreover, women in the luteal phase of their menstrual cycle (when estrogen levels are relatively higher) had a significantly lower glucose Ra and Rd at 90 min of exercise (Zderic et al. 2001; Devries et al. 2006) and lower glucose metabolic clearance rates as compared with women in the follicular phase (when estrogen levels are relatively lower) (Devries et al. 2006). Women in the luteal phase of their menstrual cycle also had lower proglycogen and macroglycogen (two pools that make up total glycogen content) utilization during exercise compared to women in the follicular phase (Devries et al. 2006). To try and understand the cellular mechanism regulating CHO metabolism, Fu et al. (2009) looked for differences in key regulatory genes—glucose transporter type 4 (GLUT-4), hexokinase 2 (HK II), phosphofructokinase (PFK), glycogenin, glycogen synthase 1 (GS-1), glycogen synthase kinase 3 alpha (GSK3α), and glycogen phosphorylase mRNA content—involved in skeletal muscle CHO metabolism between men and women, and found no coordinate or directional differences between sexes, suggesting that skeletal muscle tissue may not be directly regulating CHO metabolism.

Together, these data support the hypothesis that sex regulates at least some differences in CHO metabolism between men and women. Nonetheless, it should be noted that CHO utilization in skeletal muscle does not appear to be directly regulating the observed differences in whole-body substrate oxidation between the sexes. Rather, research suggests that the differences in whole-body substrate oxidation during endurance exercise are responding to a sex difference in fat metabolism (discussed below), and CHO utilization is simply changing to maintain metabolic balance.

CARBOHYDRATE UTILIZATION AND EXERCISE PERFORMANCE IN WOMEN ATHLETES

Most if not all athletes are familiar with the term *carbohydrate loading*. CHO loading involves increasing dietary CHO intake while minimizing training volume, approximately 3 to 4 days before an endurance event in order to maximize muscle glycogen storage, with the ultimate goal to improve endurance performance. CHO loading in men has been shown to increase muscle glycogen stores and improve endurance exercise performance at intensities from 60 to 75% of maximal aerobic power for 60 to 90 min (Bergstrom et al. 1967; Costill et al. 1981). Interestingly,

there appears to be a sex difference in the efficacy of CHO loading. When age- and fitness-matched athletic women and men increased their pre-exercise CHOs to 75% total energy intake, there was an increase in resting muscle glycogen content by 45% in men, which was correlated with a similar increase in performance (43%), whereas the females did not show an increase in glycogen content or in exercise performance (Tarnopolsky et al. 1995). Walker et al. (2000) found similar results when they studied well-trained women athletes and showed modest increases in muscle glycogen and exercise performance following 78% CHO loading (Walker et al. 2000).

It should be noted that both of the studies described above utilized a similar percentage of energy intake as opposed to a similar absolute amount of carbohydrate. Because women typically consume fewer calories than men, using a relative percentage of total energy intake (i.e., 75%) would mean that the women were consuming less total carbohydrate than the men. In the studies described above both relative and absolute energy intake were significantly different between the men and women, and thus, the women consumed less total carbohydrate as well as CHO on a per kilogram body weight basis (<7 g/kg/d) compared to men who generally consumed >8 g/kg/d (Karlsson and Saltin 1971; Sherman et al. 1981; Tarnopolsky et al. 1995). In studies where women increased their total energy intake by ~30% to get CHO intake to >8 g/kg/d (~75% of their energy from CHOs), there was a significant increase in muscle glycogen content (James et al. 2001; Paul et al. 2001; Tarnopolsky, Zawada et al. 2001; Andrews et al. 2003; McLay et al. 2007); albeit, the magnitude of the increase was only half that of men on a similar diet (James et al. 2001; Tarnopolsky, Zawada et al. 2001). Despite the increase in muscle glycogen content, endurance performance was not improved in women (Paul et al. 2001; Andrews et al. 2003; McLay et al. 2007). To achieve a CHO intake of >8 g/kg/d would require an energy intake many female athletes would likely never achieve. For example, a female athlete who weighs 60 kg would need to consume 2560 kcal/d in order to attain an intake of >8 g/kg/d (assuming 75% of her energy intake is from CHO). The practical issues of associated weight gain from increasing total energy intake may deter from this approach. Thus, the balance of the data suggests that the ability and practical issues surrounding CHO loading in women may limit the acceptance and efficacy of such a strategy, and it appears that endurance performance is not enhanced.

CHO intake can also be manipulated during endurance exercise in the post-exercise recovery period. The provision of exogenous CHO during endurance exercise can delay the onset of fatigue and promote higher glucose oxidation rates in the latter stages of endurance exercise in both women (Bailey et al. 2000; Campbell et al. 2001) and men (Coggan and Coyle 1989, 1991; Coggan and Swanson 1992; Burelle et al. 1999; Febbraio et al. 2000). Moreover, research suggests that women who use exogenous CHO during exercise may even "spare" glycogen, as compared with men (Riddell et al. 2003). In the study conducted by Riddell et al. (2003), they compared men and women during two exercise trials where the subjects cycled on an ergometer for 90 min at 60% VO_2 peak, 1 week apart, consuming either an 8% exogenous CHO drink (1 g glucose/kg/h) or a placebo (zero-calorie) drink. The proportion of energy derived from exogenous CHO (relative to lean body mass) tended to be higher in women compared to men. These data suggest that women may oxidize a greater relative proportion of exogenous

CHO during endurance exercise which, in turn, may spare endogenous CHO and prevent early glycogen depletion and premature fatigue. Taken together with the findings from Campbell, Angus et al. (2001) that 6% exogenous CHO intake, compared with placebo intake, dramatically increases cycling performance at 70% VO_{2max} in women, it is recommended that women consume exogenous CHOs during endurance exercise to improve exercise performance.

There are also significant benefits of CHO supplementation in the post-exercise recovery period in women. Post-exercise CHO supplementation (~1 g/kg of CHO immediately following endurance exercise) improves glycogen resynthesis to a similar extent in both women and men (Tarnopolsky et al. 1997). Furthermore, CHOs plus protein supplementation immediately following exercise during a week of intensified training have been shown to increase nitrogen (protein) retention and improve exercise performance in women (Roy et al. 2002).

In summary, despite the lower CHO oxidation rates in women compared to men, women can still benefit from CHO consumption around exercise. More specifically, consuming exogenous CHO during exercise has been shown to improve endurance performance in women. Similarly, CHO intake immediately post-exercise increases the rates of glycogen resynthesis in women and can spare protein utilization in women during intensified training. And, while CHO loading before an endurance exercise bout can effectively increase glycogen storage in women so long as the amount of CHO is sufficiently large (>8 g/kg/d), the risk of weight gain with no observable difference in performance makes such a recommendation questionable.

SEX DIFFERENCES IN FAT METABOLISM

Overview of Fat Metabolism

Fats become proportionately more important as a fuel source as exercise duration increases (and glycogen is depleted) and as the intensity of exercise decreases (Stanley and Connett 1991; Powers and Howley 1996). There are two primary groups of fats that contribute to energy metabolism: fatty acids (FAs) and triglycerides. FAs, specifically long-chain fatty acids (LCFAs), are an important contributor to ATP production in skeletal muscle both at rest and during low- to moderate-intensity aerobic exercise. For example, during aerobic exercise, FAs contribute anywhere from 30 to 70% of substrate utilized, depending on exercise intensity and duration (Friedlander et al. 2007), and approximately 90% of those FAs are derived from LCFAs (Havel et al. 1963). Fats are predominantly stored as triglycerides in adipocytes; however, FAs can also be stored in skeletal muscle as intramyocellular lipids (IMCLs), also known as intramyocellular triglycerides (IMTGs) (Morgan et al. 1969). IMCLs are situated in the sarcoplasma in direct contact or proximity to mitochondria, the cellular organelle responsible for substrate oxidation and energy production (Hoppeler 1986; Tarnopolsky et al. 2006), serving as a direct energy source during aerobic exercise. IMCLs are found in the greatest concentrations in oxidative type I muscle fibers (Hwang et al. 2001). Furthermore, trained athletes have a higher IMCL content compared with sedentary people, and IMCL content is lower following prolonged submaximal exercise (Staron et al. 1989).

Sex Differences in Fat Oxidation

The lower RER seen in women during steady-state endurance exercise represents higher whole-body fat oxidation as compared with men (Tarnopolsky 2008). Coordinately, women have been shown to have a higher amount of IMCL compared with men (Roepstorff et al. 2002, 2006; Tarnopolsky et al. 2007), which is attributed to more total fat droplets (hyperplasia) as opposed to larger fat droplet size (hypertrophy) (Tarnopolsky et al. 2007). Using glycerol tracers, several studies have found that women have a higher lipolytic rate as compared with men during endurance exercise (45 to 65% VO_{2max} for 60 to 90 minutes) (Friedlander et al. 1998; Carter et al. 2001a; Mittendorfer et al. 2002). Biochemical assays have also shown a greater use of IMCLs during endurance exercise in women (Roepstorff et al. 2002; Steffensen et al. 2002; Roepstorff et al. 2006). There is also evidence that women can replenish IMCL stores in a shorter duration compared with men, as trained women runners on a moderate-fat diet (35% energy) restored baseline IMCL content in 22 hours (Larson-Meyer et al. 2002), yet it took trained male cyclists 48 hours to replace IMCL content on a moderate-fat diet (39% energy) (van Loon et al. 2003), albeit, these data were derived from two separate studies.

The mechanism for the higher-fat use in women during endurance exercise is not fully understood. The intake of high-fat diets has been shown to increase fat oxidation and whole-body lipolysis by increasing the amount of IMCL in men, irrespective of changes in plasma free fatty acids (Zderic et al. 2004). However, there is no evidence that the difference in fat utilization in sex difference studies is attributed to differences in diet, as comparisons of diets in women and men have shown no significant difference in macronutrient distribution (Devries et al. 2006; Zalcman et al. 2007; Lun et al. 2009; Maher et al. 2010b). Some research suggests sex differences in fat oxidation are largely due to a greater ability to transport FAs into skeletal muscle cells and a more efficient mitochondrial oxidation capacity seen in women (Figure 1.1) (reviewed in Glatz et al. 2010). That is, women have a significantly higher abundance of membrane fatty acid transporter (FATm) mRNA (Binnert et al. 2000), plasma membrane fatty acid binding protein (FABPpm) protein and mRNA, fatty acid translocase (FAT/CD36) protein, and muscle lipoprotein lipase (mLPL) (Kiens et al. 2004), all of which should translate into a higher capacity for fatty acid transport into skeletal muscle. Women also have a higher percentage of IMCLs touching mitochondria post-exercise compared with men (Devries et al. 2007). In addition, women have a higher protein content of key enzymes involved in the mitochondrial breakdown of FAs, including very long-chain acyl-CoA dehydrogenases (VLCADs), medium-chain acyl-CoA dehydrogenases (MCADs), trifunctional protein (TFP), and long-chain hydroxyacyl-CoA dehydrogenase (HADHA) (also known as trifunctional protein α) than men (Maher et al. 2010a), suggesting that women have a higher capacity for β-oxidation of FAs. This may be due to the increased transcriptional activation of the genes responsible for fat oxidation in women (Maher et al. 2009).

There are physiological differences in muscle fiber type between women and men, which might ultimately play a role in the differences in fat oxidation. Women have a greater percent area of type I fibers than men in the *vastus lateralis* (Carter et al. 2001b; Maher et al. 2009), and men have a significantly larger type I fiber area

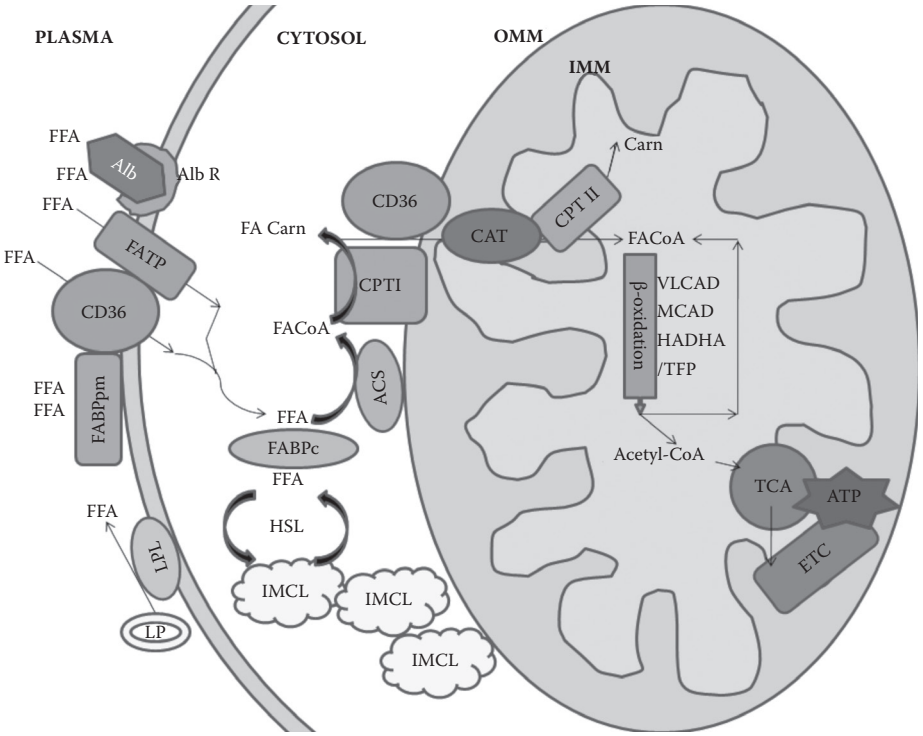


FIGURE 1.1 Fatty acid metabolism in skeletal muscle. (ACS, Acyl-CoA synthetase; Alb, albumin; ATGL, adipose triglyceride lipase; CAT, carnitine acyltransferase; CPT, carnitine palmitoyltransferase; ETC, electron transport chain; FABP, fatty acid binding protein; FATP, fatty acid transport protein; FFA, free fatty acid; HADHA/TFP, long-chain hydroxyacyl-CoA dehydrogenase/trifunctional protein; HSL, hormone-sensitive lipase; IMCL, intramyocellular lipid; IMM, inner mitochondrial membrane; LP, lipoprotein; LPL, lipoprotein lipase; MCAD, medium-chain acyl-CoA dehydrogenases; OMM, outer mitochondrial membrane; TCA, tricarboxylic acid cycle; VLCAD, very long-chain acyl-CoA dehydrogenases.)

in the *biceps brachii* and larger type II fiber area in the *vastus lateralis* than women (Miller et al. 1993; Carter et al. 2001b). Similar results were found in rats where the cross-sectional areas of type II fibers of the soleus and tibialis were greater in males, and the cross-sectional area of type I fibers was greater in females; however, there were no significant differences in the percentage of each individual fiber (Fox et al. 2003). Metabolically, a higher proportion of type I fibers has been correlated with higher fat oxidation rates. Type I fibers have a greater expression of fatty acid transport protein, FAT/CD36 (Vistisen et al. 2004), and women express almost 50% more FAT/CD36 than men (Kiens et al. 2004). Muscle size and function can be altered by exercise, nutrition, hormones, and aging, which ultimately affect fat oxidation.

In summary, research strongly supports sex differences in fat oxidation; specifically, women have higher fat oxidation during endurance exercise due to a greater and possibly more efficient uptake of plasma free FAs and oxidation of FAs than men, which likely accounts for the higher IMCL content. Based on this information

it could be surmised that sport-related dietary interventions may be different for men and women. Current studies comparing the effect of high-fat diets on exercise performance are contradictory as some demonstrate a positive outcome (Lambert et al. 1994; Rowlands and Hopkins 2002) and others no change (Phinney et al. 1983; Goedecke et al. 1999). However, one would predict that women may benefit from a moderately high fat recovery diet (35% energy), whereas men may not, and it would be of interest to determine if there is a sex difference in the responsiveness to high-fat diets between men and women athletes. Last, the ability of women to oxidize more fat during endurance exercise in combination with greater fat stores suggests that women would be ideally suited to complete long-duration endurance exercise and possibly outperform men in ultra-endurance events. In support of this hypothesis, it has been reported that women outperformed men at a distance of 85 km when matched for their marathon pace (Speechly et al. 1996).

Effects of Estrogen on CHO and Fat Utilization during Endurance Exercise

Estrogens are a group of steroid hormones and the primary sex hormone in women. There are three predominant estrogens in women: estradiol (17-β-estradiol), which is in the greatest abundance in women from menarche to menopause; estrone, which is the predominant estrogen in postmenopausal women; and estriol, which is the primary estrogen during pregnancy. Estrogens are produced from androgens through enzymatic alteration and can freely diffuse across the cell membrane where they can bind and activate estrogen receptors that modulate gene expression (Simpson 2003). Estrogens are well characterized in the regulation of reproduction, and in eumenorrheic women estradiol is highest in the late follicular phase of the menstrual cycle, specifically the week prior to ovulation (Simpson 2003). Estrogens also regulate many physiological functions of the musculoskeletal (Srivastava et al. 2001), gastrointestinal, immune (Wilder 1998), neural (McEwen and Alves 1999), and cardiovascular systems (Mendelsohn and Karas 1999).

Studies using ovariectomized rodents or oral administration of 17-β-estradiol to rodents have shown that estrogen has a major influence upon CHO metabolism at the skeletal and hepatic level (Kendrick and Ellis 1991; Rooney et al. 1993; Ruby et al. 1997; Carter, McKenzie et al. 2001). Ovariectomization, resulting in a significant reduction in circulating estradiol, caused an increase in glycogen utilization and lower fat utilization in both skeletal muscle and heart, similar to the metabolic pattern in male rats (Kendrick et al. 1987; Hatta et al. 1988). These effects were reverted back to the normal female-like metabolic pattern of higher fat and lesser glycogen utilization by supplementing the ovariectomized rats with 17-β-estradiol (Kendrick et al. 1987; Hatta et al. 1988). The sparing of muscle and liver glycogen and increase in free fatty acid use during exercise in male or ovariectomized female rats supplemented with 17-β-estradiol lead to an overall improvement in exercise performance (Kendrick and Ellis 1991; Rooney et al. 1993). 17-β-Estradiol also influences fat storage in mice by increasing intramuscular triglyceride content in both heart and skeletal muscle (Ellis et al. 1994).

In humans, women utilize less glycogen during endurance exercise compared with men (Carter et al. 2001a; Ruby et al. 2002; Devries et al. 2006), and there does

not appear to be a sex or menstrual cycle difference in basal muscle glycogen content (Tarnopolsky et al. 1997; McKenzie et al. 2000; James et al. 2001; Tarnopolsky, Roy et al. 2001). However, during endurance exercise women in the luteal phase of the menstrual cycle utilize significantly less proglycogen, macroglycogen, and total glycogen compared with the follicular phase (Devries et al. 2006). Interestingly, a female-like metabolic pattern was observed with administration of 17-β-estradiol to men. The administration of 17-β-estradiol to men increased their plasma 17-β-estradiol concentration to mid-follicular levels and lowered RER, which reflected an increase in fat metabolism similar to that observed in women (Devries et al. 2005; Maher et al. 2010). Men given 17-β-estradiol also had a reduction in the basal level of total muscle glycogen at rest and after exercise without affecting muscle glycogen breakdown during exercise (Devries et al. 2005). Similarly, administration of 17-β-estradiol in both men and women attenuated hepatic glucose production during endurance exercise (Ruby et al. 1997; Carter, McKenzie et al. 2001). Studies have also found that glucose rate of appearance and disappearance (Friedlander et al. 1998) and glucose metabolic clearance rate (Carter, McKenzie et al. 2001) were lower for exercising women as compared with men. Interestingly, men given 17-β-estradiol had lower proglycogen, total glycogen, hepatic glucose production, and glucose uptake suggesting whole-body glycogen sparing (Devries et al. 2005).

Mechanistically, estrogens elicit their effects by binding estrogen receptors (ERs) in the cells. ERs are known transcription factors for the regulation of genes, and ERα and ERβ mRNA and protein have been documented in skeletal muscle of humans (Lemoine et al. 2003; Wiik et al. 2003), rats (Lemoine et al. 2002a), and mice (Barros et al. 2006). ER expression seems to be regulated by exercise and fiber type. Specifically, ERα and ERβ expression was found to be higher in endurance-trained men compared to moderately active men (Wiik et al. 2005), and 7 weeks of exercise in female rats increased ERα mRNA expression (Lemoine et al. 2002a). In rats, ER expression is higher in slow twitch oxidative muscle than in fast twitch oxidative-glycolytic and glycolytic muscle, and the change in muscle ER expression due to training seems to be muscle type specific (Lemoine et al. 2002b). Differences in ER expression in muscle fiber type and increased expression during exercise suggests that ERs are involved in muscle adaptation to exercise, most likely acting at the level of gene regulation.

Based on physiological differences in humans, transgenic mice are being developed to help lead the way in understanding the mechanisms and other physiological-related outcomes of the effects of estrogen-based sex differences. For example, estrogen has been shown to modulate insulin sensitivity in women (Godsland 2005), possibly by altering insulin-related gene expression (Morimoto et al. 2001; von Wolff et al. 2003). A primary gene target in the regulation of insulin sensitivity is the glucose transporter GLUT-4. ERα has been shown to be a positive regulator, and ERβ a negative regulator, of GLUT-4 expression (Barros et al. 2006), and estrogen has been shown to regulate the signaling molecule inositol triphosphate (IP3) that plays a role in GLUT-4 translocation to the sarcolemma (Simoncini et al. 2000; Jessen and Goodyear 2005). When GLUT-4 was overexpressed in a transgenic murine model, there was an increase in the percent of glucose disposal through glycolysis in male animals and an increase in glycogen storage in female animals

(Tsao et al. 2001). Interestingly, estrogen receptor-α knockout mice exhibit insulin resistance (Heine et al. 2000). Another gene of interest is the peripheral peroxisome activating receptors (PPARs), which are a family of nuclear transcription factors that affect the expression of many target genes involved in metabolism, cell proliferation, and cell differentiation. An interesting sex difference was observed when male PPARα knockout mice died with severe hypoglycemia when an inhibitor (etomoxir) of carnitine palmitoyltransferase (CPT: a mitochondrial enzyme that mediates the transport of long-chain fatty acids across the mitochondrial membrane) activity was given, yet the majority of female mice survived (Djouadi et al. 1998). Administration of 17-β-estradiol to the male PPARα mice prevented the fatal effects of CPT inhibition (Djouadi et al. 1998), demonstrating the interrelatedness of glucose and fat oxidation and the relationships to 17-β-estradiol. 17-β-Estradiol also upregulated the expression of PPARδ and activated adenosine monophosphate (AMP)-activated protein kinase in mice, suggesting that estrogen promotes the partitioning of free fatty acids toward oxidation (D'Eon et al. 2005). Further investigation into the molecular mechanism of 17-β-estradiol in ovariectomized mice showed that lipogenic genes were downregulated in adipocytes, liver, and skeletal muscle (D'Eon et al. 2005).

Substrate utilization is a fine balance between CHO and fat metabolism. Women have a significantly lower RER compared with men, and men supplemented with 17-β-estradiol have a lower RER, which reflects both a lower reliance on CHOs and an increase in fat utilization, suggesting that estrogen is also acting on fat metabolism (Devries et al. 2005; Hamadeh et al. 2005; Maher et al. 2010a). Administration of 17-β-estradiol to male rats increased lipoprotein lipase (LPL) activity in skeletal muscle and decreased it in adipocytes (Ellis et al. 1994). This suggests that estrogen is involved in the preferred storage of fats in the skeletal muscle, perhaps making fats more readily available for oxidation. Similarly, women have 160% higher mRNA for LPL than men, but there were no observed sex differences in LPL activity (Kiens et al. 2004). There are significant sex differences in the expression of fat transport proteins, where women have approximately 50% higher FAT/CD36 protein and twice the amount of fatty acid transport protein (FATP)-1 mRNA compared with men (Binnert et al. 2000; Kiens et al. 2004). Women compared to age- and fitness-matched men also have higher mRNA content for FATm (Binnert et al. 2000) and FABPpm (Kiens et al. 2004). A greater abundance of fatty acid transporters at the cell membrane has been shown to correlate significantly with free fatty acid uptake into the cell (Holloway et al. 2007); however, more research is needed to determine whether or not this finding translates into functional significance in humans.

Once fats are transported into the cell they must be transported into the mitochondria to be oxidized (Figure 1.1). It is unknown if there are sex differences in mitochondrial population of FAT/CD36 that might contribute to greater mitochondrial fatty acid uptake and thus greater fat oxidation. However, women have significantly higher mRNA content of citrate synthase (Roepstorff et al. 2005), β-hydroxyacyl-CoA dehydrogenase (β-HAD) (Roepstorff et al. 2005), and hormone-sensitive lipase (Roepstorff et al. 2006). There appear to be no significant sex differences in CPT-1 or β-HAD activity in humans; however, 17-β-estradiol supplementation in ovariectomized rats leads to higher maximal enzyme activities for CPT-1 and β-HAD (Campbell and Febbraio 2001). Furthermore, women have a higher mRNA

expression of genes involved in intramyocellular fat synthesis (SREBP-1c and mtG-PAT), transcriptional regulation (PPARα and PPARδ), and β-oxidation (TFP-α) (Tarnopolsky 2008; Fu et al. 2009). There are also significant sex differences in mitochondrial β-oxidation enzymes, where women were shown to have significantly higher protein content for TFPα, VLCAD, and MCAD, suggesting that women have a greater capacity to oxidize more long-chain fatty acids during exercise. Last, 17-β-estradiol administration in men significantly increased the mRNA content of PGC-1α, PPARδ, TFPα, CPT1, SREBP-1c, mtGPAT, GLUT-4, GS-1, and AST (Tarnopolsky 2008; Fu et al. 2009) and increased the protein abundance of MCAD and TFPα (Maher et al. 2010a). Sex differences in mRNA content and limited protein expression data appear to be directionally consistent with the observed metabolic differences present during exercise, implying that fat oxidation is regulated and CHO and protein oxidation follows based on metabolic demand, which is partially due to an effect of 17β-estradiol.

SEX DIFFERENCES IN PROTEIN METABOLISM DURING EXERCISE

As previously mentioned, fats and CHOs are the preferred energy sources for both men and women during endurance exercise. Under normal circumstances amino acid oxidation only accounts for between 2 and 6% of total energy requirements (Phillips et al. 1993; McKenzie et al. 2000). If protein is to be used for energy, it must first be catabolized to its constituent amino acids and then the amino acids are deaminated (i.e., the nitrogen is removed) and the carbon skeletons oxidized (MacLean et al. 1991; Stanley and Connett 1991; Powers and Howley 1996). The nitrogen is rarely reused and instead is incorporated into urea and excreted in the urine where it can be measured as an indirect marker of protein catabolism.

Urinary urea excretion and stable isotope tracers have been used to estimate sex differences in the relative contribution of amino acid oxidation to intermediary metabolism during endurance exercise (Phillips et al. 1993; McKenzie et al. 2000; Lamont et al. 2001a,b; Riddell et al. 2003). Initial studies found that women had lower urinary nitrogen excretion following endurance exercise as compared with men (Tarnopolsky et al. 1990), indicative of a relatively lower protein oxidation. Subsequent research using ^{13}C-leucine stable isotope methodology confirmed that leucine oxidation was lower for women as compared to men during endurance exercise (<70% VO_{2max} for 60 to 90 minutes) when matched for training status (Phillips et al. 1993; Kobayashi et al. 1997; McKenzie et al. 2000; Lamont et al. 2001a, 2003).

Mechanistically, substrate availability definitely plays a role in metabolic regulation of protein oxidation (Gaine et al. 2006); however, most well-designed sex comparison studies have ensured that men and women were on controlled diets and received comparable protein based on grams per kilogram body weight. The increase in leucine oxidation could be attributable to activation of branched-chain 2-oxo acid dehydrogenase (BCOAD), the rate-limiting enzyme for muscle branched-chain amino acid oxidation. Basal activation of BCOAD was found to be lower in women than in men; however, the activation was similar between the sexes after exercise (McKenzie et al. 2000). There have been studies showing differences in amino acid kinetics during different phases of the menstrual cycle. Specifically, research

has shown that urinary urea nitrogen excretion was higher during the luteal compared to follicular phase of the menstrual cycle (Lamont et al. 1987). Coordinately, women had a higher leucine oxidation during the luteal compared with follicular phase (Lariviere et al. 1994). These findings suggest that there are sex differences in protein regulation during exercise, and these sex differences could be related to factors such as estrogen and progesterone levels. Interestingly, when men are supplemented with 17-β-estradiol for 8 days leucine oxidation during 90 min of cycling was significantly lower by ~16% (Hamadeh et al. 2005). It was found that the mRNA for branched-chain 2-oxo acid dehydrogenase kinase (BCOADK), the inactivator of BCOAD, was significantly higher for women and went up 33% in men supplemented with 17-β-estradiol, although the latter did not reach statistical significance (Fu et al. 2009).

Sex differences in protein oxidation could also be a result of variation in catecholamine responsiveness (Lamont et al. 1995, 2003). Pharmacological administration of propranalol, a catecholamine receptor blocker, further increased leucine oxidation in men yet had no effect in women (Lamont et al. 1995, 2003). These results suggest that a component of sex, likely hormone related, regulates protein oxidation. In light of these initial studies, further research is needed to determine the metabolic regulation of sex-related differences in amino acid utilization and the implications these differences might have with respect to specific protein requirements for female athletes.

SUMMARY

The majority of research that has examined sex differences in metabolic fuel selection during submaximal endurance exercise supports a higher fat oxidation and lower CHO and protein oxidation in women compared to men and that these differences are driven largely by differences in fatty acid metabolism with CHO metabolism changing to maintain metabolic energy balance. Specifically, there is strong evidence suggesting that women have greater uptake or transport of fats into the skeletal muscle, greater storage of fats as IMCL, and greater mitochondrial fat oxidation. Although the exact mechanisms underlying the differences in substrate oxidation between the sexes have not been fully elucidated, it appears as though estrogen plays a key role. The increased efficiency that women have for the use of fat appears to be primarily regulated by estrogenic hormones (estrogen and maybe progesterone) at the skeletal muscle and hepatic level. Sex-related differences exist in muscle fiber type, and it is important to consider that this may influence metabolism and adaptive responses to exercise; however, acute 17-β-estradiol administration to men shifts fuel selection toward higher fat oxidation while fiber type remains unaltered (Hamadeh et al. 2005). Thus, the role of estrogen in muscle remains an intriguing potential mechanism underlying the observed sex differences. More research is necessary to decipher these potential sex differences and how they affect muscle metabolism.

The nutritional implications of the substrate oxidation differences for the female athlete are not well defined; however, some logical suggestions can be made. First, because women have an apparent inability to increase their muscle glycogen in response to an *acute* increase in the percent of energy intake from dietary CHO (Tarnopolsky

et al. 1995), CHO loading should be done at least 4 days in advance of the endurance event by increasing both energy intake and the absolute amount of CHOs (>8 g/kg/d) (Tarnopolsky, Zawada et al. 2001). Nonetheless, it should be emphasized that the magnitude of the increase in glycogen storage in women, even under these conditions, is only approximately 50% of that seen in men, and there is little evidence of a performance benefit. Second, exogenous CHO during endurance exercise has been shown to produce the same performance advantages in women as men (Bailey et al. 2000; Campbell et al. 2001). Thus, female endurance athletes should be encouraged to follow the CHO recommendations outlined in Chapter 2. Third, women have similar muscle glycogen resynthesis responses to men when provided CHO in the early post-exercise period. Consuming CHO at a rate of 1 g/kg/h or CHO (0.8 g/kg/h) plus protein (0.3 g/kg/h) starting immediately post-exercise maximizes the rate of skeletal muscle glycogen resynthesis (reviewed in Betts and Williams 2010). And, finally, because women have a greater capacity to transport and store fats and utilize fats during exercise, women might benefit from a higher fat recovery diet (30 to 40% energy) after long duration training or racing events to replenish IMCL stores in skeletal muscle. The Acceptable Macronutrient Distribution Ranges (AMDR) recommend that 20 to 35% of an adult's dietary intake should be derived from unsaturated fats (Zello 2006). Unsaturated fats include fats found in fish, nuts, seeds, and vegetable oil (canola, corn, flaxseed, olive, peanut, soybean, and sunflower). It is important to note that high dietary fat can increase IMCL content and plays a role in decreasing insulin sensitivity in nonathletes (Goodpaster et al. 2001).

REFERENCES

Andrews, J. L., D. A. Sedlock, et al. (2003). Carbohydrate loading and supplementation in endurance-trained women runners. *J Appl Physiol* 95(2): 584–590.

Bailey, S. P., C. M. Zacher, et al. (2000). Effect of menstrual cycle phase on carbohydrate supplementation during prolonged exercise to fatigue. *J Appl Physiol* 88(2): 690–697.

Barros, R. P., U. F. Machado, et al. (2006). Muscle GLUT-4 regulation by estrogen receptors ERβ and ERα. *Proc Natl Acad Sci U S A* 103(5): 1605–1608.

Bergstrom, J., L. Hermansen, et al. (1967). Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71(2): 140–150.

Betts, J. A., and C. Williams (2010). Short-term recovery from prolonged exercise: Exploring the potential for protein ingestion to accentuate the benefits of carbohydrate supplements. *Sports Med* 40(11): 941–959.

Binnert, C., H. A. Koistinen, et al. (2000). Fatty acid transport protein-1 mRNA expression in skeletal muscle and in adipose tissue in humans. *Am J Physiol Endocrinol Metab* 279(5): E1072–E1079.

Blatchford, F. K., R. G. Knowlton, et al. (1985). Plasma FFA responses to prolonged walking in untrained men and women. *Eur J Appl Physiol Occup Physiol* 53(4): 343–347.

Brooks, S., M. E. Nevill, et al. (1990). The hormonal responses to repetitive brief maximal exercise in humans. *Eur J Appl Physiol Occup Physiol* 60(2): 144–148.

Burelle, Y., F. Peronnet, et al. (1999). Oxidation of an oral [13C]glucose load at rest and prolonged exercise in trained and sedentary subjects. *J Appl Physiol* 86(1): 52–60.

Campbell, S. E., D. J. Angus, et al. (2001). Glucose kinetics and exercise performance during phases of the menstrual cycle: Effect of glucose ingestion. *Am J Physiol Endocrinol Metab* 281(4): E817–E825.

Campbell, S. E., and M. A. Febbraio (2001). Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. *Am J Physiol Endocrinol Metab* 281(4): E803–E808.

Carter, S., S. McKenzie, et al. (2001). Short-term 17β-estradiol decreases glucose R(a) but not whole body metabolism during endurance exercise. *J Appl Physiol* 90(1): 139–146.

Carter, S. L., C. Rennie, et al. (2001a). Substrate utilization during endurance exercise in men and women after endurance training. *Am J Physiol Endocrinol Metab* 280(6): E898–E907.

Carter, S. L., C. D. Rennie, et al. (2001b). Changes in skeletal muscle in males and females following endurance training. *Can J Physiol Pharmacol* 79(5): 386–392.

Coggan, A. R., and E. F. Coyle (1989). Metabolism and performance following carbohydrate ingestion late in exercise. *Med Sci Sports Exerc* 21(1): 59–65.

Coggan, A. R., and E. F. Coyle (1991). Carbohydrate ingestion during prolonged exercise: Effects on metabolism and performance. *Exerc Sport Sci Rev* 19: 1–40.

Coggan, A. R., and S. C. Swanson (1992). Nutritional manipulations before and during endurance exercise: Effects on performance. *Med Sci Sports Exerc* 24(9 Suppl): S331–S335.

Costill, D. L., J. Daniels, et al. (1976). Skeletal muscle enzymes and fiber composition in male and female track athletes. *J Appl Physiol* 40(2): 149–154.

Costill, D. L., W. J. Fink, et al. (1979). Lipid metabolism in skeletal muscle of endurance-trained males and females. *J Appl Physiol* 47(4): 787–791.

Costill, D. L., W. M. Sherman, et al. (1981). The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. *Am J Clin Nutr* 34(9): 1831–1836.

Cureton, K. J., and P. B. Sparling (1980). Distance running performance and metabolic responses to running in men and women with excess weight experimentally equated. *Med Sci Sports Exerc* 12(4): 288–294.

D'Eon, T. M., S. C. Souza, et al. (2005). Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 280(43): 35983–35991.

Davis, S. N., P. Galassetti, et al. (2000). Effects of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. *J Clin Endocrinol Metab* 85(1): 224–230.

Devries, M. C., M. J. Hamadeh, et al. (2005). 17β-Estradiol supplementation decreases glucose rate of appearance and disappearance with no effect on glycogen utilization during moderate intensity exercise in men. *J Clin Endocrinol Metab* 90(11): 6218–6225.

Devries, M. C., M. J. Hamadeh, et al. (2008). Endurance training without weight loss lowers systemic, but not muscle, oxidative stress with no effect on inflammation in lean and obese women. *Free Radic Biol Med* 45(4): 503–511.

Devries, M. C., M. J. Hamadeh, et al. (2006). Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *Am J Physiol Regul Integr Comp Physiol* 291(4): R1120–R1128.

Devries, M. C., S. A. Lowther, et al. (2007). IMCL area density, but not IMCL utilization, is higher in women during moderate-intensity endurance exercise, compared with men. *Am J Physiol Regul Integr Comp Physiol* 293(6): R2336–R2342.

Djouadi, F., C. J. Weinheimer, et al. (1998). A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor α-deficient mice. *J Clin Invest* 102(6): 1083–1091.

Ellis, G. S., S. Lanza-Jacoby, et al. (1994). Effects of estradiol on lipoprotein lipase activity and lipid availability in exercised male rats. *J Appl Physiol* 77(1): 209–215.

Febbraio, M. A., A. Chiu, et al. (2000). Effects of carbohydrate ingestion before and during exercise on glucose kinetics and performance. *J Appl Physiol* 89(6): 2220–2226.

Ferrando, A. A., M. Sheffield-Moore, et al. (2002). Testosterone administration to older men improves muscle function: Molecular and physiological mechanisms. *Am J Physiol Endocrinol Metab* 282(3): E601–E607.

Fox, J., P. Garber, et al. (2003). Morphological characteristics of skeletal muscles in relation to gender. *Aging Clin Exp Res* 15(3): 264–269.

Friedlander, A. L., G. A. Casazza, et al. (1998). Training-induced alterations of carbohydrate metabolism in women: Women respond differently from men. *J Appl Physiol* 85(3): 1175–1186.

Friedlander, A. L., K. A. Jacobs, et al. (2007). Contributions of working muscle to whole body lipid metabolism are altered by exercise intensity and training. *Am J Physiol Endocrinol Metab* 292(1): E107–E116.

Froberg, K., and P. K. Pedersen (1984). Sex differences in endurance capacity and metabolic response to prolonged, heavy exercise. *Eur J Appl Physiol Occup Physiol* 52(4): 446–450.

Fu, M. H., A. C. Maher, et al. (2009). Exercise, sex, menstrual cycle phase, and 17β-estradiol influence metabolism-related genes in human skeletal muscle. *Physiol Genomics* 40(1): 34–47.

Gaine, P. C., M. A. Pikosky, et al. (2006). Level of dietary protein impacts whole body protein turnover in trained males at rest. *Metabolism* 55(4): 501–507.

Glatz, J. F., J. J. Luiken, et al. (2010). Membrane fatty acid transporters as regulators of lipid metabolism: Implications for metabolic disease. *Physiol Rev* 90(1): 367–417.

Godsland, I. F. (2005). Oestrogens and insulin secretion. *Diabetologia* 48(11): 2213–2220.

Goedecke, J. H., C. Christie, et al. (1999). Metabolic adaptations to a high-fat diet in endurance cyclists. *Metabolism* 48(12): 1509–1517.

Goedecke, J. H., A. St Clair Gibson, et al. (2000). Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *Am J Physiol Endocrinol Metab* 279(6): E1325–E1334.

Gollnick, P. D., K. Piehl, et al. (1974). Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *J Physiol* 241(1): 45–57.

Goodpaster, B. H., J. He, et al. (2001). Skeletal muscle lipid content and insulin resistance: Evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86(12): 5755–5761.

Hamadeh, M. J., M. C. Devries, et al. (2005). Estrogen supplementation reduces whole body leucine and carbohydrate oxidation and increases lipid oxidation in men during endurance exercise. *J Clin Endocrinol Metab* 90(6): 3592–3599.

Hatta, H., Y. Atomi, et al. (1988). The effects of ovarian hormones on glucose and fatty acid oxidation during exercise in female ovariectomized rats. *Horm Metab Res* 20(10): 609–611.

Havel, R. J., A. Naimark, et al. (1963). Turnover rate and oxidation of free fatty acids of blood plasma in man during exercise: Studies during continuous infusion of palmitate-1-C14. *J Clin Invest* 42: 1054–1063.

Heine, P. A., J. A. Taylor, et al. (2000). Increased adipose tissue in male and female estrogen receptor-α knockout mice. *Proc Natl Acad Sci USA* 97(23): 12729–12734.

Holloway, G. P., J. Lally, et al. (2007). Fatty acid binding protein facilitates sarcolemmal fatty acid transport but not mitochondrial oxidation in rat and human skeletal muscle. *J Physiol* 582(Pt 1): 393–405.

Hoppeler, H. (1986). Exercise-induced ultrastructural changes in skeletal muscle. *Int J Sports Med* 7(4): 187–204.

Horton, T. J., G. K. Grunwald, et al. (2006). Glucose kinetics differ between women and men, during and after exercise. *J Appl Physiol* 100(6): 1883–1894.

Horton, T. J., M. J. Pagliassotti, et al. (1998). Fuel metabolism in men and women during and after long-duration exercise. *J Appl Physiol* 85(5): 1823–1832.

Hwang, J. H., J. W. Pan, et al. (2001). Regional differences in intramyocellular lipids in humans observed by *in vivo* 1H-MR spectroscopic imaging. *J Appl Physiol* 90(4): 1267–1274.

James, A. P., M. Lorraine, et al. (2001). Muscle glycogen supercompensation: Absence of a gender-related difference. *Eur J Appl Physiol* 85(6): 533–538.
Jensen, M. D., P. E. Cryer, et al. (1996). Effects of epinephrine on regional free fatty acid and energy metabolism in men and women. *Am J Physiol* 270(2 Pt 1): E259–E264.
Jessen, N., and L. J. Goodyear (2005). Contraction signaling to glucose transport in skeletal muscle. *J Appl Physiol* 99(1): 330–337.
Kang, J., S. L. Rashti, et al. (2009). Effect of preceding resistance exercise on metabolism during subsequent aerobic session. *Eur J Appl Physiol* 107(1): 43–50.
Karlsson, J., and B. Saltin (1971). Diet, muscle glycogen, and endurance performance. *J Appl Physiol* 31(2): 203–206.
Kendrick, Z. V., and G. S. Ellis (1991). Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats. *J Appl Physiol* 71(5): 1694–1699.
Kendrick, Z. V., C. A. Steffen, et al. (1987). Effect of estradiol on tissue glycogen metabolism in exercised oophorectomized rats. *J Appl Physiol* 63(2): 492–496.
Kiens, B., C. Roepstorff, et al. (2004). Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: Influence of physical activity and gender. *J Appl Physiol* 97(4): 1209–1218.
Kobayashi, R., Y. Shimomura, et al. (1997). Gender difference in regulation of branched-chain amino acid catabolism. *Biochem J* 327 (Pt 2): 449–453.
Lambert, E. V., D. P. Speechly, et al. (1994). Enhanced endurance in trained cyclists during moderate intensity exercise following 2 weeks adaptation to a high fat diet. *Eur J Appl Physiol Occup Physiol* 69(4): 287–293.
Lamont, L. S. (2005). Gender differences in amino acid use during endurance exercise. *Nutr Rev* 63(12 Pt 1): 419–422.
Lamont, L. S., P. W. Lemon, et al. (1987). Menstrual cycle and exercise effects on protein catabolism. *Med Sci Sports Exerc* 19(2): 106–110.
Lamont, L. S., A. J. McCullough, et al. (1995). Beta-adrenergic blockade heightens the exercise-induced increase in leucine oxidation. *Am J Physiol* 268(5 Pt 1): E910–E916.
Lamont, L. S., A. J. McCullough, et al. (2001a). Gender differences in leucine, but not lysine, kinetics. *J Appl Physiol* 91(1): 357–362.
Lamont, L. S., A. J. McCullough, et al. (2001b). Relationship between leucine oxidation and oxygen consumption during steady-state exercise. *Med Sci Sports Exerc* 33(2): 237–241.
Lamont, L. S., A. J. McCullough, et al. (2003). Gender differences in the regulation of amino acid metabolism. *J Appl Physiol* 95(3): 1259–1265.
Lariviere, F., R. Moussalli, et al. (1994). Increased leucine flux and leucine oxidation during the luteal phase of the menstrual cycle in women. *Am J Physiol* 267(3 Pt 1): E422–E428.
Larson-Meyer, D. E., B. R. Newcomer, et al. (2002). Influence of endurance running and recovery diet on intramyocellular lipid content in women: A 1H NMR study. *Am J Physiol Endocrinol Metab* 282(1): E95–E106.
Lemoine, S., P. Granier, et al. (2002a). Effect of endurance training on oestrogen receptor α transcripts in rat skeletal muscle. *Acta Physiol Scand* 174(3): 283–289.
Lemoine, S., P. Granier, et al. (2002b). Effect of endurance training on oestrogen receptor α expression in different rat skeletal muscle type. *Acta Physiol Scand* 175(3): 211–217.
Lemoine, S., P. Granier, et al. (2003). Estrogen receptor α mRNA in human skeletal muscles. *Med Sci Sports Exerc* 35(3): 439–443.
Lun, V., K. A. Erdman, et al. (2009). Evaluation of nutritional intake in Canadian high-performance athletes. *Clin J Sport Med* 19(5): 405–411.
M'Kaouar, H., F. Peronnet, et al. (2004). Gender difference in the metabolic response to prolonged exercise with [13C]glucose ingestion. *Eur J Appl Physiol* 92(4–5): 462–469.
MacLean, D. A., L. L. Spriet, et al. (1991). Plasma and muscle amino acid and ammonia responses during prolonged exercise in humans. *J Appl Physiol* 70(5): 2095–2103.

Maher, A. C., M. Akhtar, et al. (2010a). Men supplemented with 17β-estradiol have increased β-oxidation capacity in skeletal muscle. *Physiol Genomics* 42(3): 342–347.

Maher, A. C., M. Akhtar, et al. (2010b). Women have higher protein content of β-oxidation enzymes in skeletal muscle than men. *PLoS One* 5(8): e12025.

Maher, A. C., M. H. Fu, et al. (2009). Sex differences in global mRNA content of human skeletal muscle. *PLoS ONE* 4(7): e6335.

McEwen, B. S., and S. E. Alves (1999). Estrogen actions in the central nervous system. *Endocr Rev* 20(3): 279–307.

McKenzie, S., S. M. Phillips, et al. (2000). Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans. *Am J Physiol Endocrinol Metab* 278(4): E580–E587.

McLay, R. T., C. D. Thomson, et al. (2007). Carbohydrate loading and female endurance athletes: Effect of menstrual-cycle phase. *Int J Sport Nutr Exerc Metab* 17(2): 189–205.

Melanson, E. L., T. A. Sharp, et al. (2002). Effect of exercise intensity on 24-h energy expenditure and nutrient oxidation. *J Appl Physiol* 92(3): 1045–1052.

Mendelsohn, M. E., and R. H. Karas (1999). The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 340(23): 1801–1811.

Miller, A. E., J. D. MacDougall, et al. (1993). Gender differences in strength and muscle fiber characteristics. *Eur J Appl Physiol Occup Physiol* 66(3): 254–262.

Mittendorfer, B., J. F. Horowitz, et al. (2002). Effect of gender on lipid kinetics during endurance exercise of moderate intensity in untrained subjects. *Am J Physiol Endocrinol Metab* 283(1): E58–E65.

Monjo, M., A. M. Rodriguez, et al. (2003). Direct effects of testosterone, 17β-estradiol, and progesterone on adrenergic regulation in cultured brown adipocytes: Potential mechanism for gender-dependent thermogenesis. *Endocrinology* 144(11): 4923–4930.

Morgan, T. E., F. A. Short, et al. (1969). Effect of long-term exercise on skeletal muscle lipid composition. *Am J Physiol* 216(1): 82–86.

Morimoto, S., M. A. Cerbon, et al. (2001). Insulin gene expression pattern in rat pancreas during the estrous cycle. *Life Sci* 68(26): 2979–2985.

Nicklas, B. J., A. C. Hackney, et al. (1989). The menstrual cycle and exercise: Performance, muscle glycogen, and substrate responses. *Int J Sports Med* 10(4): 264–269.

Paul, D. R., S. M. Mulroy, et al. (2001). Carbohydrate-loading during the follicular phase of the menstrual cycle: Effects on muscle glycogen and exercise performance. *Int J Sport Nutr Exerc Metab* 11(4): 430–441.

Phillips, S. M., S. A. Atkinson, et al. (1993). Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J Appl Physiol* 75(5): 2134–2141.

Phinney, S. D., B. R. Bistrian, et al. (1983). The human metabolic response to chronic ketosis without caloric restriction: Preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism* 32(8): 769–776.

Pillard, F., C. Moro, et al. (2007). Lipid oxidation according to intensity and exercise duration in overweight men and women. *Obesity (Silver Spring)* 15(9): 2256–2262.

Powers, S. K., and Howley, E.T. (1996). *Exercise Physiology, Theory and Application to Fitness and Performance.* New York: WCB/McGraw-Hill.

Ramis, J. M., R. Salinas, et al. (2006). Depot- and gender-related differences in the lipolytic pathway of adipose tissue from severely obese patients. *Cell Physiol Biochem* 17(3-4): 173–180.

Riddell, M. C., S. L. Partington, et al. (2003). Substrate utilization during exercise performed with and without glucose ingestion in female and male endurance trained athletes. *Int J Sport Nutr Exerc Metab* 13(4): 407–421.

Roepstorff, C., M. Donsmark, et al. (2006). Sex differences in hormone-sensitive lipase expression, activity, and phosphorylation in skeletal muscle at rest and during exercise. *Am J Physiol Endocrinol Metab* 291(5): E1106–E1114.

Roepstorff, C., P. Schjerling, et al. (2005). Regulation of oxidative enzyme activity and eukaryotic elongation factor 2 in human skeletal muscle: Influence of gender and exercise. *Acta Physiol Scand* 184(3): 215–224.

Roepstorff, C., C. H. Steffensen, et al. (2002). Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. *Am J Physiol Endocrinol Metab* 282(2): E435–E447.

Roepstorff, C., M. Thiele, et al. (2006). Higher skeletal muscle α2AMPK activation and lower energy charge and fat oxidation in men than in women during submaximal exercise. *J Physiol* 574(Pt 1): 125–138.

Romijn, J. A., E. F. Coyle, et al. (2000). Substrate metabolism during different exercise intensities in endurance-trained women. *J Appl Physiol* 88(5): 1707–1714.

Rooney, T. P., Z. V. Kendrick, et al. (1993). Effect of estradiol on the temporal pattern of exercise-induced tissue glycogen depletion in male rats. *J Appl Physiol* 75(4): 1502–1506.

Rowlands, D. S., and W. G. Hopkins (2002). Effects of high-fat and high-carbohydrate diets on metabolism and performance in cycling. *Metabolism* 51(6): 678–690.

Roy, B. D., K. Luttmer, et al. (2002). The influence of post-exercise macronutrient intake on energy balance and protein metabolism in active females participating in endurance training. *Int J Sport Nutr Exerc Metab* 12(2): 172–188.

Ruby, B. C., A. R. Coggan, et al. (2002). Gender differences in glucose kinetics and substrate oxidation during exercise near the lactate threshold. *J Appl Physiol* 92(3): 1125–1132.

Ruby, B. C., R. A. Robergs, et al. (1997). Effects of estradiol on substrate turnover during exercise in amenorrheic females. *Med Sci Sports Exerc* 29(9): 1160–1169.

Sherman, W. M., D. L. Costill, et al. (1981). Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. *Int J Sports Med* 2(2): 114–118.

Simoncini, T., A. Hafezi-Moghadam, et al. (2000). Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. *Nature* 407(6803): 538–541.

Simpson, E. R. (2003). Sources of estrogen and their importance. *J Steroid Biochem Mol Biol* 86(3–5): 225–230.

Skinner, J. S., A. Jaskolski, et al. (2001). Age, sex, race, initial fitness, and response to training: The HERITAGE Family Study. *J Appl Physiol* 90(5): 1770–1776.

Speechly, D. P., S. R. Taylor, et al. (1996). Differences in ultra-endurance exercise in performance-matched male and female runners. *Med Sci Sports Exerc* 28(3): 359–365.

Spriet, L. L., and S. J. Peters (1998). Influence of diet on the metabolic responses to exercise. *Proc Nutr Soc* 57(1): 25–33.

Srivastava, S., G. Toraldo, et al. (2001). Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-κ B ligand (RANKL)-induced JNK activation. *J Biol Chem* 276(12): 8836–8840.

Stanley, W. C., and R. J. Connett (1991). Regulation of muscle carbohydrate metabolism during exercise. *Faseb J* 5(8): 2155–2159.

Staron, R. S., R. S. Hikida, et al. (1989). Lipid depletion and repletion in skeletal muscle following a marathon. *J Neurol Sci* 94(1–3): 29–40.

Steffensen, C. H., C. Roepstorff, et al. (2002). Myocellular triacylglycerol breakdown in females but not in males during exercise. *Am J Physiol Endocrinol Metab* 282(3): E634–E642.

Tarnopolsky, L. J., J. D. MacDougall, et al. (1990). Gender differences in substrate for endurance exercise. *J Appl Physiol* 68(1): 302–308.

Tarnopolsky, M. A. (2000). Gender differences in substrate metabolism during endurance exercise. *Can J Appl Physiol* 25(4): 312–327.

Tarnopolsky, M. A. (2008). Sex differences in exercise metabolism and the role of 17-β estradiol. *Med Sci Sports Exerc* 40(4): 648–654.

Tarnopolsky, M. A., S. A. Atkinson, et al. (1995). Carbohydrate loading and metabolism during exercise in men and women. *J Appl Physiol* 78(4): 1360–1368.

Tarnopolsky, M. A., M. Bosman, et al. (1997). Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. *J Appl Physiol* 83(6): 1877–1883.

Tarnopolsky, M. A., C. D. Rennie, et al. (2006). The influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. *Am J Physiol Regul Integr Comp Physiol*.

Tarnopolsky, M. A., C. D. Rennie, et al. (2007). Influence of endurance exercise training and sex on intramyocellular lipid and mitochondrial ultrastructure, substrate use, and mitochondrial enzyme activity. *Am J Physiol Regul Integr Comp Physiol* 292(3): R1271–R1278.

Tarnopolsky, M. A., B. D. Roy, et al. (2001). Short-term 17-β-estradiol administration does not affect metabolism in young males. *Int J Sports Med* 22(3): 175–180.

Tarnopolsky, M. A., and W. H. Saris (2001). Evaluation of gender differences in physiology: An introduction. *Curr Opin Clin Nutr Metab Care* 4(6): 489–492.

Tarnopolsky, M. A., C. Zawada, et al. (2001). Gender differences in carbohydrate loading are related to energy intake. *J Appl Physiol* 91(1): 225–230.

Tate, C. A., and R. W. Holtz (1998). Gender and fat metabolism during exercise: A review. *Can J Appl Physiol* 23(6): 570-582.

Timmons, B. W., M. J. Hamadeh, et al. (2005). Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling. *J Appl Physiol* 99(3): 979–985.

Tsao, T. S., J. Li, et al. (2001). Metabolic adaptations in skeletal muscle overexpressing GLUT-4: Effects on muscle and physical activity. *Faseb J* 15(6): 958–969.

van Loon, L. J., V. B. Schrauwen-Hinderling, et al. (2003). Influence of prolonged endurance cycling and recovery diet on intramuscular triglyceride content in trained males. *Am J Physiol Endocrinol Metab* 285(4): E804–E811.

Vistisen, B., K. Roepstorff, et al. (2004). Sarcolemmal FAT/CD36 in human skeletal muscle colocalizes with caveolin-3 and is more abundant in type 1 than in type 2 fibers. *J Lipid Res* 45(4): 603–609.

von Wolff, M., S. Ursel, et al. (2003). Glucose transporter proteins (GLUT) in human endometrium: Expression, regulation, and function throughout the menstrual cycle and in early pregnancy. *J Clin Endocrinol Metab* 88(8): 3885–3892.

Walker, J. L., G. J. Heigenhauser, et al. (2000). Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. *J Appl Physiol* 88(6): 2151–2158.

Wallis, G. A., R. Dawson, et al. (2006). Metabolic response to carbohydrate ingestion during exercise in males and females. *Am J Physiol Endocrinol Metab* 290(4): E708–E715.

Wiik, A., B. Glenmark, et al. (2003). Oestrogen receptor β is expressed in adult human skeletal muscle both at the mRNA and protein level. *Acta Physiol Scand* 179(4): 381–387.

Wiik, A., T. Gustafsson, et al. (2005). Expression of oestrogen receptor α and β is higher in skeletal muscle of highly endurance-trained than of moderately active men. *Acta Physiol Scand* 184(2): 105–112.

Wilder, R. L. (1998). Hormones, pregnancy, and autoimmune diseases. *Ann NY Acad Sci* 840: 45–50.

Wolfe, R., A. Ferrando, et al. (2000). Testosterone and muscle protein metabolism. *Mayo Clin Proc* 75 Suppl: S55–S59; discussion S59–S60.

Zalcman, I., H. V. Guarita, et al. (2007). Nutritional status of adventure racers. *Nutrition* 23(5): 404–411.

Zderic, T. W., A. R. Coggan, et al. (2001). Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *J Appl Physiol* 90(2): 447–453.

Zderic, T. W., C. J. Davidson, et al. (2004). High-fat diet elevates resting intramuscular triglyceride concentration and whole body lipolysis during exercise. *Am J Physiol Endocrinol Metab* 286(2): E217–E225.

Zehnder, M., M. Ith, et al. (2005). Gender-specific usage of intramyocellular lipids and glycogen during exercise. *Med Sci Sports Exerc* 37(9): 1517–1524.

Zello, G. A. (2006). Dietary Reference Intakes for the macronutrients and energy: Considerations for physical activity. *Appl Physiol Nutr Metab* 31(1): 74–79.

2 Carbohydrates Requirements for the Female Athlete

Louise M. Burke and Christine E. Dziedzic

CONTENTS

CARBOHYDRATE REQUIREMENTS FOR THE FEMALE ATHLETE

Research has consistently demonstrated the importance of consuming adequate amounts of carbohydrate for the optimal training and performance of the endurance athlete. The amount of carbohydrate required as well as the timing of carbohydrate intake is likely as unique as the athlete. As such, recommendations for carbohydrate intakes are now made to athletes with specificity for the type of sport, the volume (i.e., intensity, duration) of training, and the athlete's competition goals and schedule. Whether carbohydrate recommendations should also be tailored to the sex (gender) of the athlete remains a topic of great interest. The specificity of such advice could be based on potential sex-based differences in carbohydrate metabolism during exercise (see Chapter 1 for more information on sex differences in substrate utilization). Alternatively or additionally, it could take into account that women must meet their carbohydrate requirements from a different dietary framework than male athletes; for example, to choose carbohydrate-rich foods that can simultaneously address

increased requirements for other nutrients while achieving a lower total energy requirement. Finally, advice to females might need to consider differences in the practical or psychological aspects underpinning food choices that differ between the sexes.

The aim of this chapter is to review the current evidence pertaining to the carbohydrate needs of female athletes. More specifically, the current guidelines for the carbohydrate requirements of sport will be described, noting recommendations for total carbohydrate intake over a day as well as specific needs before, during, and after exercise. Evidence that these guidelines apply to females equally well as to men will be presented. This will include an examination of whether the female reproductive hormones alter carbohydrate metabolism during exercise in a sufficiently robust way that justifies an alteration to strategies for carbohydrate intake. Finally, examples will be provided of ways in which females can address their carbohydrate intake goals in the light of other nutritional needs, including balancing carbohydrate needs with energy concerns, requirements for other nutrient needs, and the pursuit of a healthy relationship with food.

UPDATES ON CARBOHYDRATE REQUIREMENTS FOR ATHLETES

The importance of carbohydrate as a substrate for exercise, particularly endurance exercise, has formed a major principle of sports nutrition since the pioneering work of Scandinavian exercise scientists (Ahlborg et al. 1967; Bergstrom et al. 1967). We know that carbohydrate availability to the muscle and central nervous system can be compromised, both in training and during competition, because the fuel cost of an exercise program can often exceed the body's capacity to store carbohydrate. Strategies to provide adequate carbohydrate are important because carbohydrate availability is a limiting factor in the performance of prolonged sessions (>90 min) of submaximal or intermittent high-intensity exercise and plays a permissive role in the performance of brief or sustained high-intensity work. Such strategies include recovery of muscle glycogen stores between exercise sessions, specific fueling practices in the hours and days before a competition event, and the intake of carbohydrate during exercise.

A recent meeting of the International Olympic Committee (IOC) on Nutrition for Sport provided an opportunity to update the guidelines for practices to meet the carbohydrate requirements of exercise (Burke et al. 2011). A key proposal from this meeting was to change the *qualitative* terminology used to describe the carbohydrate content of an athlete's diet. That is, rather than use subjective labels of *high-carbohydrate diets* or *low-carbohydrate diets*, it was determined that an individual assessment be made of how well an athlete's carbohydrate intake matches the fuel needs of his or her specific training program—that is, *carbohydrate availability*. The balance sheet would compare an athlete's total daily carbohydrate intake and the timing of its consumption in relation to training against the fuel cost of training and event commitments. Does the eating plan maintain an adequate supply of carbohydrate substrate for the muscle and central nervous system during exercise (*high carbohydrate availability*) or are carbohydrate fuel sources depleted or limiting for the daily exercise program (*low carbohydrate availability*) (Burke et al.

2011)? This approach would mean that two athletes who consume the same amount of carbohydrate might differ in the assessment of how suitable these intakes are, or that an athlete with a relatively low intake of carbohydrate might still be considered to have high carbohydrate availability when measured against the fuel needs of her sport. These guidelines were an extension of earlier recommendations that *quantitative* descriptions of carbohydrate intake be changed—specifically, that carbohydrate targets should be provided in grams relative to an athlete's body mass (g/kg) rather than its contribution to total energy intake (% energy) (Burke et al. 2004). The new carbohydrate guidelines expand the targets for an adequate carbohydrate supply for exercise from those recommended in 2004 (see Table 2.1) with the continued caveat that these should be fine-tuned with individual consideration of total energy needs, specific training needs, and feedback from training performance. The need for different recommendations for carbohydrate intake based on sex was not considered within these guidelines due to the lack of specific information on this issue. Hence, one of the goals of the present chapter is to specifically consider how well these guidelines apply to females. In addition, it was noted that the expansion of daily targets for carbohydrate intake relied on theoretical and speculative estimates of the carbohydrate cost of different types of exercise rather than direct measurements of fuel utilization during a range of sporting activities (Burke et al. 2011).

Several other concepts regarding targets for carbohydrate intake were remodeled in the most recent IOC update. It was emphasized that an athlete's carbohydrate needs are not fixed but rather change according to daily, weekly, or seasonal goals and exercise commitments in a periodized training program (Burke et al. 2011). Therefore, an athlete might target a carbohydrate intake from any of the "ranges" noted in Table 2.1 according to the actual training load of the day. In this way, athletes are encouraged to adjust their daily carbohydrate intakes by adopting eating patterns in which meals and snacks providing carbohydrate and other important nutrients are placed strategically around exercise sessions. This would mean that extra food and fluid would be automatically consumed when the athlete undertakes a training session, but not on days of rest or light training. Such a strategy allows total nutrient and energy intake to track with the changes in the fuel cost of the athlete's training commitments. In addition, the enhanced carbohydrate availability for key training sessions should enable better performance and recovery. Consideration of the type, timing, and frequency of intake of carbohydrate-rich food and fluids over the day can help to promote refueling as well as meet the athlete's overall nutrition goals. A summary of the updated guidelines for carbohydrate intake over the day, together with the evidence upon which these guidelines are based, is provided in Table 2.2.

Carbohydrate intake during exercise is another topic in which the evolution of science and practice has created sufficient support for a change in the recommendations. Until recently, carbohydrate replacement guidelines during exercise favored a simplistic "one-size-fits-all" approach. Specifically, for training sessions or events of moderate-to-high intensity lasting greater than 60 min, athletes were encouraged to target carbohydrate intakes of 30 to 60 g/h in conjunction with adequate hydration (Coyle 2004; ACSM et al. 2007). These recommendations were largely based on research indicating that carbohydrate oxidation rates approximate 1 g/min, and

TABLE 2.1
Carbohydrate Intake Targets for Athletes

	Situation	Carbohydrate Targets	Comments on Type and Timing of Carbohydrate Intake
Daily Needs for Fuel and Recovery (These general recommendations should be fine-tuned with individual consideration of total energy needs, specific training needs, and feedback from training performance.)			
Light	Low intensity or skill-based activities	3–5 g/kg of athlete's body mass/d	Timing of intake may be chosen to promote speedy refueling or to provide fuel intake around training sessions in the day. Otherwise, as long as total fuel needs are provided, the pattern of intake may simply be guided by convenience and individual choice.
Moderate	Moderate exercise program (i.e., ~1 hour per day)	5–7 g/kg/d	
High	Endurance program (e.g., 1–3 h/d moderate- to high-intensity exercise)	6–10 g/kg/d	Protein- and nutrient-rich carbohydrate food or food combinations will allow the athlete to meet other acute or chronic sports nutrition goals.
Very high	Extreme commitment (i.e., >4–5 h/d moderate- to high-intensity exercise)	8–12 g/kg/d	
Acute Fueling Strategies (These guidelines promote high carbohydrate availability to promote optimal performance in competition or key training sessions.)			
General fueling up	Preparation for events <90 min exercise	7–12 g/kg per 24 hours as for daily fuel needs	Athletes may choose compact carbohydrate-rich sources that are low in fiber or residue and easily consumed to ensure that fuel targets are met and to meet goals for gut comfort or lighter "racing weight."
Carbohydrate loading	Preparation for events >90 min of sustained or intermittent exercise	36–48 hours of 10–12 g/kg per 24 hours	

Speedy refueling	<8 hours recovery between two fuel-demanding sessions	1–1.2 g/kg/h for first 4 hours, then resume daily fuel needs	There may be benefits in consuming small, regular snacks. Compact carbohydrate-rich food and drinks may help to ensure that fuel targets are met.
Pre-event fueling	Before exercise (>60 min)	1–4 g/kg consumed 1–4 hours before exercise	Timing, amount, and type of carbohydrate food and drinks should be chosen to suit the practical needs of the event and individual preferences and experiences. Choices high in fat, protein, and fiber may need to be avoided to reduce risk of gastrointestinal issues during the event. Low glycemic index choices may provide a more sustained source of fuel for situations where carbohydrate cannot be consumed during exercise.
During brief exercise	<45 min	Not needed	
During sustained high-intensity exercise	45–75 min	Small amounts including mouth rinse	A range of drinks and sports products can provide easily consumed carbohydrate.
During endurance exercise including "stop and start" sports	1–2.5 h	30–60 g/h	Opportunities to consume food and drinks vary according to the rules and nature of each sport. A range of everyday dietary choices and specialized sports products ranging in form from liquid to solid may be useful. The athlete should practice to find a refueling plan that suits his or her individual goals including hydration needs and gut comfort.
During ultra-endurance exercise	>2.5–3 hours	Up to 90 g/h	As above. Higher intakes of carbohydrate are associated with better performance. Products providing multiple transportable carbohydrates (glucose:fructose mixtures) will achieve high rates of oxidation of carbohydrate consumed during exercise.

Source: Taken from Burke, L. M., Hawley, J. A., Wong S., and Jeukendrup, A. E. 2011, Carbohydrates for Training and Competition, *Journal of Sports Sciences* 29(Suppl. 1): S17–S27. With permission.

TABLE 2.2
Evidence Underpinning the Guidelines for Carbohydrate Intake in Athletes' Everyday Diet and for Refueling after Exercise

Guidelines for Carbohydrate Intake	Supporting Evidence
When it is important to train hard or with high intensity, daily carbohydrate intakes should match the fuel needs of training and glycogen restoration.	Although some studies fail to show benefits (Sherman et al. 1993; Cox et al. 2010), perhaps in part due to methodological issues (see Burke 2010), others show that when carbohydrate intake is higher and better matched to muscle fuel needs, the athlete can train harder (Costill et al. 1988; Simonsen et al. 1991) or perform better at the end of an intensive training block than a trial or group consuming a lower carbohydrate intake (Simonsen et al. 1991; Achten et al. 2004).
Targets for daily carbohydrate intake are usually based on body mass (or proxy for the volume of active muscle) and exercise load. Guidelines can be suggested (Table 2.1) but need to be fine-tuned according to the athlete's overall dietary goals and feedback from training.	The carbohydrate demands of exercise are determined by the volume and intensity of the workload. There is a direct relationship between the quantity of dietary carbohydrate and post-exercise glycogen storage, at least until the muscle storage capacity or threshold has been reached (Costill et al. 1981).
Guidelines for carbohydrate intake should not be provided in terms of percentage contributions to total dietary energy intake.	Guidelines based on percent (%) energy intake are not easily translated into practice. More importantly, they are not strongly related to the muscle's fuel needs based on body size and workload. In fact, in females the relationship between carbohydrate expressed as percent of energy and grams per kilogram body mass is trivial (Burke et al. 2001).
When the period between exercise sessions is <8 hours, the athlete should consume carbohydrate as soon as practical after the first workout to maximize the effective recovery time between sessions.	Glycogen storage is most rapid when carbohydrate is consumed in the hours immediately after exercise, but most importantly, in the absence of carbohydrate intake, refueling is ineffective (Ivy et al. 1988).
Early post-exercise recovery may be enhanced by a higher rate of carbohydrate intake, especially when consumed in frequent small feedings.	The highest rates of glycogen synthesis have been reported during the 0- to 4-hour period of recovery when high rates of carbohydrate intake are consumed in serial feedings (van Hall et al. 2000; van Loon et al. 2000).
During longer recovery periods (24 hours) when the athlete can consume adequate energy and carbohydrate, the types, pattern, and timing of carbohydrate-rich meals and snacks can be chosen according to what is practical and enjoyable.	When conditions support optimal refueling, there is no difference in glycogen synthesis when liquid or solid forms of carbohydrate are consumed (Keizer et al. 1986), or if carbohydrate targets are spread over large meals or frequent smaller snacks (Costill et al. 1981; Burke et al. 1996). When there is adequate time for refueling, it does not appear to matter if carbohydrate intake is delayed for a couple of hours (Parkin et al. 1997) to suit issues of food availability or appetite.

TABLE 2.2 *(Continued)*
Evidence Underpinning the Guidelines for Carbohydrate Intake in Athletes' Everyday Diet and for Refueling after Exercise

Guidelines for Carbohydrate Intake	Supporting Evidence
Carbohydrate-rich food with a moderate to high glycemic index (GI) provide a readily available source of substrate for glycogen synthesis. This may be important in situations where maximum glycogen storage is required in the hours after an exercise bout.	Foods with a low GI appear to be less effective in promoting glycogen storage (Burke et al. 1993). This may be partly due to poor digestibility that overestimates actual carbohydrate intake (Joszi et al. 1996) and may be compensated for by additional intake of these foods, or the addition of food with a high GI to meals and snacks.
Adequate energy intake is needed to optimize glycogen storage; the restrained eating practices of some athletes interferes both with meeting targets for carbohydrate intake and optimizing glycogen storage from this intake.	When energy intake is restricted, lower glycogen storage may be expected from the same carbohydrate intake (Tarnopolsky et al. 2001). This may be acceptable at times when nutrition goals require a reduced energy intake. However, good energy availability should be prioritized when efficient refueling is required.
High-quality protein sources should be included in post-exercise recovery meals and snacks to promote muscle protein synthesis. Enhanced glycogen storage may be a secondary benefit.	Protein consumed during recovery may promote glycogen storage when carbohydrate intake is suboptimal (for review, see Betts and Williams 2010), especially during the first hours of recovery (Ivy et al. 2002).
Nutrient-rich carbohydrate food or other food added to recovery meals and snacks can provide a good source of protein and other nutrients.	Other nutrients may be important in recovery processes and should also be consumed during the recovery period. Most importantly, these choices will enable the athlete to integrate all his or her nutrition goals within the same eating plan.
Athletes should follow sensible practices regarding alcohol intake at all times, but particularly in the recovery period after exercise.	Excessive intake of alcohol after exercise may directly inhibit glycogen storage during the period of elevated blood alcohol concentration. However, the most important effects of alcohol intake on refueling (and other recovery issues) is via a reduced ability, or interest, to achieve dietary goals such as the optimal amount and timing of carbohydrate intake (Burke et al. 2003).

Source: Expanded from Burke, L. M., Hawley, J. A., Wong S., and Jeukendrup, A. E. 2011, Carbohydrates for Training and Competition, *Journal of Sports Sciences* 29(Suppl. 1): S17–S27. With permission.

thus, consumption of that amount of carbohydrate would optimize oxidation rates. It is well known, however, that consuming carbohydrate during exercise can enhance performance via a number of different and overlapping mechanisms; these include the provision of an additional muscle fuel source when glycogen stores become depleted, muscle glycogen sparing, prevention of low blood glucose concentrations, and effects on the central nervous system (Karelis et al. 2010). A variety of

mechanisms would argue for a more systematic approach to meeting specific carbohydrate needs during training and athletic competitions of different duration and intensity. As highlighted in Table 2.1, there are at least three different approaches to meeting carbohydrate replacement goals during exercise.

Although there is no exact line of demarcation, sports that involve more than ~3 hours of sustained moderate- to high-intensity exercise become increasingly reliant on carbohydrate from exogenous sources as muscle stores become depleted. Despite this, previous guidelines for carbohydrate intake were capped at 60 g/h due to the prevailing belief that larger amounts of carbohydrates would cause gastrointestinal distress or require drink concentrations that compromised fluid delivery. Anecdotally, these guidelines appear to have been largely ignored by several successful endurance athletes, as surveys of such athletes have noted high intakes (~90 g/h) of carbohydrate during multiday cycling events (Saris et al. 1989) and Ironman triathlons (Kimber et al. 2002). The answer to achieving these rates has been recently found through a series of studies that systematically tracked the oxidation rates of various sources, forms, and combinations of carbohydrate consumed during exercise (Jeukendrup 2010). This work found that the rate-limiting step in the oxidation of ingested carbohydrate is its intestinal absorption, with the sodium-dependent glucose transporter SGLT1 limiting the absorption of glucose in its various forms to ~1 g/min. However, when glucose is consumed in combination with a carbohydrate that uses a different absorption mechanism (e.g., fructose, using GLUT-5 transporter), rates of ingested carbohydrate can exceed 1.5 g/min (Jentjens et al. 2004, 2006).

Several studies using these carbohydrate combinations (known as *multiple transportable carbohydrates*) at such rates have shown benefits to the performance of exercise activities of ~3 hours duration compared with the ingestion of glucose alone (Currell and Jeukendrup 2008; Triplett et al. 2010). Furthermore, evidence of a dose-response relationship between carbohydrate intake and performance of such events is emerging in which the optimal rate of intake appears to be within the range of 60 to 90 g/h (Smith et al. 2010a, 2010b). Therefore, the new guidelines for ultra-endurance sport promote individual experimentation with mixtures of carbohydrates of up to 90 g/h (see Table 2.1). It should be noted that guidelines for carbohydrate intake during exercise are provided in absolute amounts, because there is little difference in the oxidation of exogenous carbohydrate according to body size or body mass (Jeukendrup 2010). Instead, factors such as the carbohydrate content of the habitual diet or intake during training sessions (Cox et al. 2010) may play a role in determining capacity for oxidizing carbohydrate ingested during exercise.

Sports involving ~1 hour of sustained or intermittent high-intensity exercise are at the other end of the spectrum of activities that can benefit from carbohydrate intake during the session. Because such exercise is not limited by the availability of muscle glycogen stores given adequate nutritional preparation, the early reports of enhanced performance associated with carbohydrate intake were perplexing (for review, see Burke et al. 2005). Findings of a lack of improvement of a 1-hour cycling protocol with glucose *infusion* (Carter et al. 2004b) but benefits from carbohydrate *ingestion* (Jeukendrup et al. 1997) created an intriguing hypothesis that the central nervous system might sense the presence of carbohydrate via receptors in the mouth and oral

space, promoting an enhanced sense of well-being and improved pacing. This theory was subsequently confirmed by observations that simply rinsing the mouth with a carbohydrate solution can also enhance performance of the cycling bout (Carter et al. 2004a). There is now robust evidence that in situations when a high-power output is required over durations of ~45 to 75 minutes (e.g., the road cycling time trial, half-marathons), mouth rinsing or intake of very small amounts of carbohydrate play a largely nonmetabolic role involving the central nervous system in enhancing performance by 2 to 3% (Jeukendrup and Chambers 2010). These findings have been incorporated into updated guidelines for carbohydrate intake during exercise (Table 2.1).

The Concept of "Training Low"

Research has investigated the potential ergogenic benefits of deliberately training with low carbohydrate availability ("training low") and then subsequently competing with high carbohydrate availability. The idea for such a practice was born out of studies examining the cellular response to exercise in a glycogen-depleted state, which demonstrated that an acute bout of (endurance) exercise commenced with low muscle glycogen results in a greater transcriptional activation of enzymes involved in carbohydrate metabolism and an increase in adaptive responses favoring fat metabolism (see Hawley et al. 2011). A much-publicized investigation provided some limited evidence to support apparent benefits of this training technique (Hansen et al. 2005). In this study, previously sedentary men ($n = 7$) underwent a 10-week training program consisting of knee extensor exercises in which one leg was trained once per day (high glycogen condition) while the other leg trained twice every other day (low glycogen condition). The results showed a greater increase in knee extensor endurance in the low glycogen condition. These findings were accompanied by a greater increase in the maximal activity of metabolic enzymes citrate synthase and HAD, and were attributed to the commencement of 50% of the training sessions with a low glycogen concentration. Although these findings have significant scientific merit and possible application for exercise programs targeting metabolic improvements and health outcomes, there are some concerns in applying the technique to the endurance athlete interested in sports performance.

The first problem is the misconception that "train-low" techniques require chronic adherence to a low-carbohydrate diet. In fact, the protocol undertaken by Hansen and colleagues used the placement of training sessions within a carbohydrate-rich diet to achieve low glycogen levels for a selected proportion of workouts. Other ways to selectively reduce carbohydrate availability for some training sessions include exercising after an overnight fast, consuming water during prolonged workouts, withholding carbohydrates in the hours after exercise, or restricting carbohydrate below the fuel requirements of the training load (Burke 2010). Different techniques can manipulate the duration of exposure to a low-carbohydrate environment as well as the focus on reducing endogenous and exogenous carbohydrate stores. The second problem is trying to equate findings of "clamped" training programs (training at the same power-specific output for each session) in untrained people to athletes who undertake periodized training programs involving progressive overload and

self-pacing (Hawley and Burke 2010). In fact, studies utilizing the "two a day training" model of low glycogen training in trained populations have failed to find any enhancement of the performance gains from training (Yeo et al. 2008; Hulston et al. 2010). Although there is consistent evidence that undertaking some exercise sessions with low glycogen/exogenous carbohydrate availability can enhance the metabolic adaptations associated with training, even in well-trained individuals, changes in muscle physiology are not a proxy for performance per se. Currently there is no convincing evidence that train-low strategies achieve an enhancement of performance over a conventional diet and training approach (Hawley and Burke 2010). Furthermore, several disadvantages are associated with train-low techniques including an impairment of the ability to train at high intensities; this is significant because it is a cornerstone of the principles of preparation for elite sport (Burke 2010).

In summary, further research on this topic is needed, but a pragmatic commentary on practices in the field is that athletes already periodize the carbohydrate availability for their training sessions. By design or by accident, some workouts are undertaken with reduced carbohydrate availability (e.g., the second or third session of a day during high-volume periods, early morning sessions undertaken before breakfast, training during a period of energy restriction for weight loss), while others are undertaken with good carbohydrate support (quality sessions scheduled during lower-volume periods, sessions undertaken after a meal). Thus, the real question is not whether there is a role for dietary periodization with carbohydrate availability, but whether it should be exploited in a different way (Burke et al. 2011). For the moment, athletes should focus on good carbohydrate availability for sessions requiring high intensity or high levels of technique and skill, while noting that it is less important during lower-intensity workouts or the conditioning sessions at the beginning of a season.

Is There a Need for Sex-Specific Carbohydrate Requirements in Sport?

The guidelines reviewed in the above paragraphs provide universal recommendations for the carbohydrate needs of athletes without specific adjustment for the sex of the individual athlete. The absence of sex-specific guidelines could mean that females do not have special considerations regarding carbohydrate metabolism during exercise or carbohydrate requirements for sport. Alternatively, this could mean that there is no information on which the effect of sex can be based. It is of importance to differentiate between these two options because the absence of evidence for an effect is not the same as evidence for the absence of an effect.

It is clear from examining the data on which guidelines for carbohydrate are based that the majority of research has involved male subjects. Females are underrepresented in sports nutrition research both as part of a mixed-sex study cohort or as a separate arm or focus of study. There are a number of possible reasons for the paucity of data on female athletes and strategies of carbohydrate intake for sport. Many researchers find that it is easier to recruit male athletes for research projects. This may be due to their greater rates of participation at the desired level in sports of interest or a better culture of willingness to be involved in research projects, particularly studies involving invasive techniques or highlighting competitiveness between

subjects. Equally, the logistics of conducting studies involving females incur a considerable additional challenge because of the real or perceived need to standardize for menstrual phase or status. Studies that directly compare the effects of carbohydrate metabolism or intake between males and females are hampered by the lack of uniformity or rationale for choosing the physiological factors on which the groups are matched. For example, should males and females be matched according to their training history and volume, their aerobic capacity or peak power outputs relative to body mass or fat free mass, their performance compared to the best in their field, or other variables? Nevertheless, further studies are needed.

The literature examining carbohydrate needs for sports performance in female athletes can be separated into three broad categories: (1) studies in which female subjects have been included within the group outcomes without distinguishing any differences based on sex; (2) studies that focused on female subjects alone, and (3) research in which direct comparisons have been made between the responses of male and female subjects to an intervention involving carbohydrate intake for sport. Studies that fall into each of these categories have been reviewed to find evidence that females can benefit from following the current recommendations regarding carbohydrate and sports performance.

Evidence of Benefits of Following Carbohydrate Recommendations by Female Athletes

There is some evidence that females benefit from high carbohydrate availability in their training diets. A cohort of rowers, including 12 males and 10 females, were divided into two groups who consumed a daily intake of either 10 g/kg or 5 g/kg carbohydrate over 4 weeks of training (Simonsen et al. 1991). This study showed that the group who trained with the higher carbohydrate intake made a greater gain in mean power achieved in repeat 2500 m ergometer trials over the duration of the study than the moderate carbohydrate group, with no gender differences being reported. O'Keeffe and colleagues (1989) studied female cyclists who completed an endurance protocol at 80% of VO_{2max} following a week of low (13% of energy, 1.2 g/kg of body weight), medium (54% of energy), or high (72% of energy, ~6 to 7 g/kg of body weight) carbohydrate (LCHO, MCHO, HCHO, respectively) intakes. They reported that mean exercise time to fatigue increased with increasing dietary carbohydrate content (LCHO: 60 ± 12 min < MCHO: 98 ± 13 and HCHO: 113 ± 28) (O'Keeffe et al. 1989). In another study, elite female hockey players were blindly administered either a carbohydrate drink (1 g/kg body mass) or a flavored placebo four times a day during an intense 7-day training camp (Kreider et al. 1995). Pre- and post-training camp physiological testing showed that those supplemented with additional carbohydrate had a greater improvement in time to maximal exhaustion, while the performance of athletes receiving the placebo drink actually declined. Additionally, the carbohydrate group reported significantly less post-training psychological fatigue.

In contrast to the findings above, no differences in time to exhaustion or ratings of perceived exertion (RPE) were detected when female endurance cyclists consumed isocaloric diets of either 3, 5, or 8 g/kg carbohydrate for 6 days before an exercise

trial during the midfollicular phase of the menstrual cycle (Dolins et al. 2003). While cyclists appeared to adhere to dietary prescription on the low- and moderate-carbohydrate diets, analysis of food records revealed that participants' actual carbohydrate intake on the high-carbohydrate diet was below what was assigned (i.e., 6.5 g/kg of body weight rather than the prescribed 8 g/kg of body weight). This may provide some reasoning for the lack of effect seen between the low and high carbohydrate intakes on exercise performance but also highlights the potential difficulty for females to meet certain carbohydrate targets recommended for sport.

The effects of carbohydrate loading were investigated in six well-trained female endurance athletes during the luteal phase of the menstrual cycle (Walker et al. 2000). After random assignment to either 7 days of a moderate-carbohydrate diet (~48% total energy from carbohydrate) or 3 days of a moderate-carbohydrate diet followed by 4 days of a high-carbohydrate diet (~78% carbohydrate), both with an exercise taper, participants completed a cycle to exhaustion. Pre-exercise muscle glycogen content increased by 13% in those who had the higher carbohydrate intake, and this was also associated with an increase in cycle time to volitional exhaustion. A study of eight endurance-trained females examined the combined effects of carbohydrate loading and supplementation on a 24.2 km self-paced treadmill performance run (Andrews et al. 2003). Participants completed three trials: (1) carbohydrate loading (73% total energy from carbohydrate for 4 days prior to the run) along with carbohydrate supplementation during exercise (6% carbohydrate solution; 6 ml/kg of body weight before, and 3 ml/kg of body weight every 20 min throughout the trial), (2) carbohydrate supplementation during exercise only (as described above), or (3) neither carbohydrate loading nor supplementation (placebo). Despite the large differences in carbohydrate intake among the three groups, there were no differences between the groups in performance time. The authors note that with a small sample size the study was likely to be underpowered to detect a performance difference. The significantly higher respiratory exchange ratio (RER) seen in the carbohydrate loading and supplementation trials, however, indicated that when carbohydrate availability is increased in trained women runners, carbohydrate is preferentially utilized during exercise.

Other studies investigating the effects of carbohydrate ingestion before and during exercise have included females in subject pools (Kern et al. 2007). For example, one study found performance improved by 2.3% compared to a placebo trial when a carbohydrate drink (14 ml/kg of body weight of a 7.6% solution) was consumed throughout a cycling time trial by 17 endurance trained males and 2 female cyclists (Jeukendrup et al. 1997). In this case, no significant differences between male and female subjects were noted, and the results were seen to apply equally to females.

Evidence for Sex Differences Regarding Carbohydrate Needs and Responses

Differences in the hormonal environment, variation in skeletal muscle mass and enzyme activity, and sex-specific demands to support metabolism in nonskeletal muscle tissue (uterine/placental) suggest that carbohydrate metabolism during exercise may, in fact, vary between males and females. However, studies that directly compare carbohydrate metabolism or strategies to alter carbohydrate availability during exercise between males and females are sparse and challenged by

methodological limitations. One of the first studies to investigate potential sex differences in the response to carbohydrate loading reported that females were not responsive to this sports nutrition protocol (Tarnopolsky et al. 1995). The study design required male and female athletes to increase the carbohydrate content of their self-reported habitual intakes from 60 to 75% of energy for a 4-day period. The men significantly increased muscle glycogen concentration by 41% in response to this dietary manipulation and achieved a 45% increase in cycling time to exhaustion at 85% VO_2 peak. Meanwhile, the women did not show significant changes in either glycogen concentration (0%) or cycling time to exhaustion (5%) and were shown to oxidize significantly more lipid and less carbohydrate during submaximal exercise compared with the men. These data would seem to indicate that carbohydrate loading is not an effective strategy for increasing glycogen storage or improving performance in female athletes. However, a subsequent study by the same research group employing a slightly different protocol found vastly different results (Tarnopolsky et al. 2001). Specifically, it found that the failure to alter glycogen storage in female athletes is underpinned by differences in absolute and relative carbohydrate and energy intakes rather than inherent sex-specific mechanisms (see Figure 2.1). When fed comparable amounts of carbohydrate relative to lean body mass, there appears to be no sex-related difference in the ability to supercompensate muscle glycogen levels (James et al. 2001). Similarly, a study comparing the effect of isocaloric carbohydrate (1 g/kg) or carbohydrate-protein-fat (0.75 g/kg, 0.1 g/kg, 0.02 g/kg, respectively) supplementation on glycogen repletion after exercise illustrated no significant difference in muscle glycogen recovery rates between men and women (Tarnopolsky et al. 1997).

Potential differences in the metabolic response to carbohydrate ingestion during exercise have been examined in moderately endurance-trained men and women using tracer techniques (Wallis et al. 2006). Both groups were shown to increase

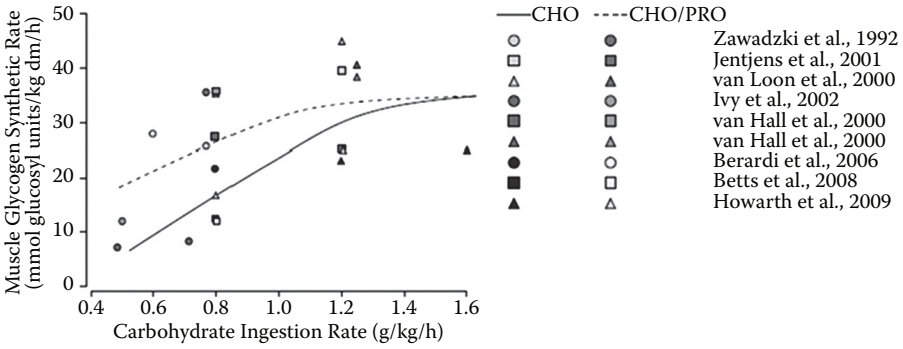


FIGURE 2.1 Reported rates of muscle glycogen resynthesis across nine studies that have compared muscle glycogen storage over >2 to 6 hours post-exercise with varied rates of carbohydrate (CHO) intake, with or without co-ingestion with protein (PRO). All studies have matched either for energy intake or carbohydrate intake. (Taken from Betts, J. A., and Williams, C., 2010, Short-Term Recovery from Prolonged Exercise: Exploring the Potential for Protein Ingestion to Accentuate the Benefits of Carbohydrate Supplements, *Sports Medicine* 40: 941–959. With permission.)

rates of appearance and disappearance of plasma glucose when fed carbohydrate during submaximal exercise, with the contribution of plasma glucose oxidation to substrate use during exercise increasing equally from about 10 to 20%. Maximal rates of exogenous carbohydrate oxidation and endogenous carbohydrate sparing in trained females have been observed when carbohydrate is ingested at a rate of 1 g/min (60 g/h) during exercise (Wallis et al. 2007). These results are consistent with the idea that the current recommendations for carbohydrate intake during exercise are suitable for female athletes and do not need to be altered according to sex.

EFFECT OF CHANGES IN FEMALE REPRODUCTIVE HORMONES ON CARBOHYDRATE REQUIREMENTS

There is a predictable fluctuation of ovarian hormones, estrogen and progesterone, over the course of a woman's menstrual cycle. Because these hormones influence metabolism and substrate utilization at rest and during exercise (see Chapter 1), there may be potential effects on the female athlete's carbohydrate requirements and carbohydrate intake strategies, which change over the course of a menstrual cycle, or as a result of the absence of the menstrual cycle, or due to the use of ovarian hormones via the oral contraceptive pill (OCP). Table 2.3 summarizes results of some of the studies that have used tracer and biopsy methodologies to measure substrate use and kinetics in each of these various situations.

Although the literature appears to show effects on carbohydrate utilization during exercise associated with different hormonal environments, it should be stressed that many of these findings are overridden by the substantial effects of factors such as differences in the intensity of exercise or the intake of carbohydrate before and during exercise (see Oosthuyse and Bosch 2010). We might expect some differences between athletes due to the interindividual variability in hormone fluxes during the menstrual cycle and the differences in the hormone concentrations of various types of OCP. Probably the most interesting difference in carbohydrate metabolism associated with female reproductive hormones lies with the observations of different capacity for glycogen storage and utilization according to the phase of the menstrual cycle. Several studies have reported that the luteal phase is associated with greater glycogen storage than the follicular phase (Nicklas et al. 1989; McLay et al. 2007). However, this difference can be restored simply by increasing the effort to carbohydrate load during the follicular phase. Whether differences in glycogen storage across phases of the menstrual cycle lead to performance differences is unclear. Further research is needed before there is sufficient evidence to address whether strategies to manipulate carbohydrate availability need to be systematically altered according to a female's hormonal status.

INTERACTIONS OF CARBOHYDRATE GOALS WITH ENERGY INTAKE

Studies examining the energy and nutrient intakes of female athletes commonly conclude that females report lower energy intakes, even when corrected for their lower body mass, than their male counterparts. For example, a summary of the dietary

TABLE 2.3
Summary of Carbohydrate Metabolism under Conditions of Different Hormonal Environments in Female Athletes

Issue	Eumenorrhea	Oral Contraceptive Pill (OCP)	Amenorrhea
Summary of condition	Normal menstrual cycle characterized by changes in hormone concentrations Luteal phase: increased circulating levels of estrogen and progesterone Follicular phase: suppression of progesterone and late surge of estrogen	Main form of birth control Varieties include combined OCP (estrogen and progesterone) or mini pill (progesterone only). Steroid doses may be monophasic, biphasic, or triphasic throughout 4-week course.	Absence of a menstrual period in a woman of reproductive age Characterized by lower serum estradiol concentration
Blood glucose utilization during exercise	Decreased* (Campbell et al. 2001; Carter et al. 2001; Zderic et al. 2001; D'Eon et al. 2002; Devries et al. 2006)	Decreased for both monophasic OCP (Bemben et al. 1992) and triphasic OCP (Suh et al. 2003)	Increased (?) (Ruby et al. 1997) (This was indirectly assumed because administration of estrogen decreased glucose Ra and Rd.)
Utilization of carbohydrate consumed during exercise	Negligible effect* (Campbell et al. 2001)		
Muscle glycogen utilization during exercise	Decreased* (Hackney 1999; Zderic et al. 2001)	Decreased for monophasic OCP (Bemben et al. 1992)	Negligible effect (Ruby et al. 1997)
Muscle glycogen resynthesis or performance benefits of carbohydrate loading	Increased* (Hackney 1990; McLay et al. 2007)	Negligible effect (Devries et al. 2006)	

* Comparison of luteal phase with follicular phase.

survey literature published between 1990 and 2000 found that female endurance athletes reported mean daily intakes of 9.42 MJ, representing 172 kJ/kg, while the equivalent values for male endurance athletes were 15.13 MJ and 227 kJ/kg (Burke et al. 2001). The mean energy intake reported by athletes involved in nonendurance events was 7.56 MJ and 125 kJ/kg for females, and 14.13 MJ and 183 kJ/kg for males, respectively. Of course, the limitations inherent in dietary survey methodology and the lack of data on training volumes add some uncertainty to the conclusions that can

be made from these observations. However, sports dietitians will attest that energy intake is a major concern for female athletes, with many individuals following restricted intakes for significant portions of the training calendar. This has several implications for carbohydrate goals and intake. The most immediate one is that the potential for achieving adequate carbohydrate intake under conditions of inadequate energy intake is decreased. The previously described review of the dietary survey literature noted that female endurance athletes reported a mean daily carbohydrate intake of 5.7 g/kg in their training diets compared to 7.6 g/kg for males. The findings for nonendurance-trained athletes were 4.5 g/kg and 5.8 g/kg for females and males, respectively. Depending on training loads, there is some likelihood that female athletes may not meet their refueling goals as effectively as males.

Strategies to Enhance Glycogen Storage from a Given Carbohydrate Intake

Although guidelines generally promote the amount of carbohydrate as the main dietary factor to manipulate to promote glycogen synthesis, in situations where total carbohydrate intake is restricted, athletes might look to strategies to enhance glycogen storage from a given or suboptimal amount of carbohydrate. There are apparently a number of opportunities to do this. For example, the use of a specifically manufactured glucose polymer with very long glucose chains (Piehl Aulin et al. 2000), the co-ingestion of large amounts of caffeine (9 mg/kg, equivalent to about 9 cups coffee) (Pedersen et al. 2008), and prior creatine loading (Robinson et al. 1999; van Loon et al. 2004) have all been shown, in male subjects at least, to increase glycogen storage from a given amount of carbohydrate. Even if these findings also apply to females, the practical implications of these strategies need to be considered. For example, the reliance on significant amounts of a glucose polymer to provide a substantial proportion of total energy intake will reduce the nutrient density of the diet and may reduce the athlete's ability to meet other nutritional goals, while the negative impact of large doses of caffeine (9 mg/kg) include interference with sleep as well as side effects such as tremors and elevated heart rates. Even if further studies can show that the glycogen storage effects of caffeine occur at lower doses, individual sensitivity may prevent it from being routinely used in such a manner. Equally, side effects associated with creatine use, such as weight gain, may mean that some athletes may not want to take advantage of any benefits on refueling strategies. Future research may identify if there are situations or individuals who can utilize the enhanced glycogen storage associated with these or other strategies. For example, they may be important in some competition situations where glycogen supercompensation or enhanced refueling during brief recovery periods may make glycogen storage a priority over other issues. Further research is needed.

The most useful strategy to increase glycogen storage from a suboptimal intake of carbohydrate, at least in the hours immediately after an exercise session, is to add protein to the recovery meal or snack. The effect of co-ingestion of protein and carbohydrate on glycogen synthesis has been a topic of debate for the past decade. The results of a recent review, however, provide a unifying explanation to the divergent results of the many studies (Betts and Williams 2010). This analysis found that when carbohydrate intake is suboptimal (<1 g/kg/h), the addition of protein increases

post-exercise glycogen storage (Figure 2.1). By contrast, when carbohydrate targets are achieved, there is minimal effect of protein intake on refueling. Of course, the main reason for consuming protein in post-exercise meals is for its stimulatory effect on the protein synthesis involved in recovery and adaptation (see Chapter 3).

Effects of Low Energy Intakes on Glycogen Storage

It is important to recognize that restricted energy intake or low energy availability have an independent effect on glycogen synthesis other than the effect on restricting total carbohydrate intake. This was clearly shown by Tarnopolsky and coworkers (2001) in a follow-up of their earlier study that appeared to show that females were less responsive to carbohydrate loading strategies than male athletes (Tarnopolsky et al. 1995). In the latter, glycogen stores in well-trained males and females were monitored following three 4-day dietary periods. As shown in Figure 2.2, when subjects consumed a diet in which the carbohydrate contribution to their habitual energy intake was increased from ~55% of energy to 75% of energy, only the males showed higher muscle glycogen stores than that achieved by their habitual diets. Although this diet featured a higher total dietary carbohydrate intake than habitual levels in both groups, the energy and carbohydrate intakes of the females were lower than the males, even when expressed relative to body mass. Only when total energy intake was increased by one third did the females show an increase in glycogen storage.

This finding has major relevance to many female athletes who may compromise muscle glycogen storage even when total carbohydrate intakes represent a high proportion

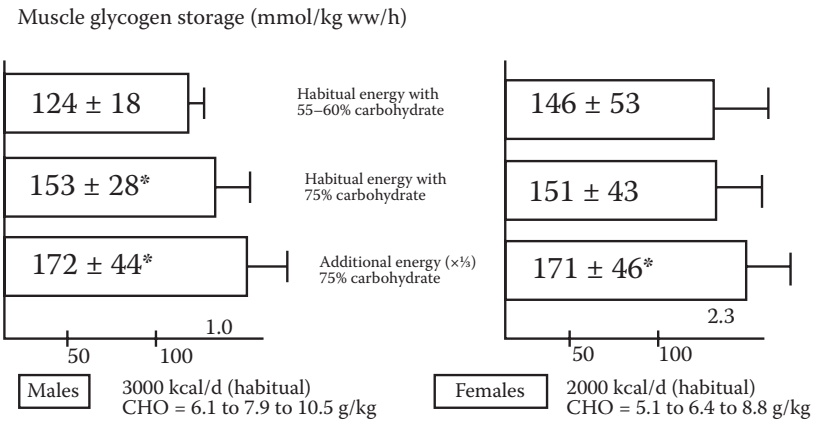


FIGURE 2.2 Glycogen stores were measured in well-trained males and females after three different dietary periods, each lasting 4 days. An increase in carbohydrate intake within habitual energy intake was associated with higher glycogen stores in male athletes but not female athletes. Only when energy intake was increased by one third did female athletes achieve an increase in dietary carbohydrate intake. (* Indicates different to baseline values.) (Drawn from Tarnopolsky, M. A., Zawada, C., Richmond, L. B., Carter, S., Shearer, J., Graham, T., and Phillips, S. M., 2001, Gender Differences in Carbohydrate Loading Are Related to Energy Intake, *Journal of Applied Physiology* 91: 225–230. With permission.)

of total energy intake or appear to meet g/kg targets. This represents another disadvantage of low energy availability and has been paraphrased by Anne Loucks, whose work underpins our understanding of this dietary phenomenon: "Females can store glycogen like men if they eat like men" (personal communication, Anne Loucks, 2006).

Periodization: A Pragmatic Approach

Female athletes often face the challenge of how to achieve the targets for carbohydrate intake to support their training load while staying within their smaller energy intakes. Even when all strategies are used to increase energy and carbohydrate intake to their maximal potential, it may still not be possible for female athletes to refuel adequately. The pragmatic approach for such females is to periodize their nutrition goals and their dietary intakes. Within the macrocycle of the training year, there may be phases where the priority is given to physique goals at the expense of energy/carbohydrate intake (e.g., the early part of the conditioning season) while the priority during other periods is to fuel optimally for performance (e.g., competition periods). Of course, even within the microcycle or training week, the athlete may choose important training days or key workouts to benefit from a higher carbohydrate intake. This plan should be individualized to each athlete and fine-tuned with experience.

FEMALE ATHLETES AND THE CULTURE OF DIETARY CARBOHYDRATE CHOICES

The final section of this chapter will focus on the characteristics of carbohydrate-rich food and food combinations that may be of particular value in constructing meal plans for female athletes. This is not based on scientific evidence that particular foods provide a physiological advantage to female athletes in comparison to males. Instead, it is based on the authors' experience that particular carbohydrate-rich foods can be useful in helping female athletes meet their overall nutrient needs and nutritional goals while addressing their exercise-based carbohydrate targets. In addition, some ideas based on considerations of common food preferences of females and their relationship with food are presented.

Themes and practices that have been observed in working with female athletes are summarized in Table 2.4 and are based on two different issues. The first issue is focused on the weight management concerns and lower energy intakes of female athletes. Here it is useful for carbohydrate-rich food or eating practices to be characterized as low to moderate in total energy value and energy density (energy value per 100 g of food), high in protein and key micronutrients (to allow other nutritional goals to be met simultaneously), and satiating (to prevent hunger or to reduce risks of overeating). A variety of carbohydrate-rich food or food combinations can address these goals (see Table 2.4 for examples). The second issue concerns common food preferences that the authors have noted among female athletes in Australia, which often revolve around the enjoyment of specific foods and the inclusion of special foods or social eating occasions in dietary practices. These examples can, at best, be described as anecdotal, and it is suggested that research be undertaken to better

TABLE 2.4
Characteristics of Carbohydrate-Rich Foods and Food Combinations That May Be of Particular Value in Menus for Female Athletes

Issue	Feature	Comments	Examples
Issues related to lower energy requirements and weight management issues: meeting nutritional goals from lower energy intakes (compared with males)	Low fat content	A low or reduced fat content reduces the energy content of the food or meal supplying a targeted amount of carbohydrate.	Low-fat, sweetened yogurt Whole-grain cereal and low-fat milk Whole-grain bread Rice or pasta
	Low energy density/ high satiety (fullness causing end of meal)	High water and fiber content allow a large volume of food to be consumed to provide a targeted carbohydrate serving.	Fresh fruit Thick vegetable soup and bread Sandwich with thick salad filling High vegetable content stir fry or casserole with noodles/rice
	Enhanced satiety (reduced hunger until next meal)	Low glycemic index carbohydrate choices may increase satiety. Co-ingestion of protein also increases the satiety of meals or snacks (see section below regarding protein).	Rolled oats—porridge or Bircher muesli Multigrained or sourdough breads Sweetened dairy products Baked beans on toast Lentil curry/casserole Noodles/pasta/quinoa/basmati rice
	High nutrient density	Valuable source of high-quality protein	Low-fat, sweetened yogurt Whole-grain cereal and low-fat milk Eggs on toast
		Valuable source of iron	Sandwich with lean beef filling Pasta with lean beef bolognese sauce
		Valuable source of calcium	Low-fat, sweetened yogurt Whole-grain cereal and low-fat milk Reduced-fat cheese on a pizza
		Valuable source of antioxidants	Fresh fruit Thick vegetable soup and bread Dried fruit and nut mix (almonds/walnuts)

TABLE 2.4 *(Continued)*
Characteristics of Carbohydrate-Rich Foods and Food Combinations That May Be of Particular Value in Menus for Female Athletes

Issue	Feature	Comments	Examples
Enhanced food enjoyment	Social eating opportunities	Many females enjoy the opportunity to consume meals and snacks in a special surrounding or a social eating situation rather than consume energy simply to meet a specific nutritional goal.	Skim-milk, hot chocolate, or frappé drink in a café Muffin or fruit bread in a café Dessert in a restaurant
	Observed preferences	Many female athletes appear to prefer sweet forms of carbohydrate in preference to savory carbohydrate-rich choices. They also prefer solid forms of carbohydrate to carbohydrate-rich fluids (e.g., sodas, sports drinks). The exception to this may be chocolate-flavored milk, but this is also preferred as a hot drink or a frozen drink (frappé), especially when consumed in a social setting (see above).	Confectionery or sports confectionery (and water) instead of sports drinks during exercise Low-fat, dairy food rather than protein supplement for post-exercise recovery Low-fat, sweetened yogurt or dairy dessert (e.g., custard or fromage frais) rather than flavored milk Carbohydrate-moderated main meal plus carbohydrate-rich dessert rather than carbohydrate-rich main meal (e.g., pasta- or rice-dominated meal) "Diet soda" and carbohydrate-rich snack rather than sweetened sodas

understand the importance of culture, sex, and dietary restraint in determining the food choices of female athletes.

SUMMARY

Female athletes have been underrepresented in studies of carbohydrate metabolism during exercise and the effect of strategies to promote carbohydrate availability on the performance of exercise and sport. Nevertheless, the available evidence suggests that the present guidelines for carbohydrate intake in the daily training and competition eating practices of athletes are suitable for female athletes. Further research should be undertaken to confirm if females have special requirements for carbohydrate intake or should use different strategies for consuming carbohydrate before, during, or after exercise to optimize performance. Some fine-tuning of these strategies might be suited to different phases of the menstrual cycle or to athletes who are amenorrheic, due to the effects of differing levels of estrogen and progesterone on carbohydrate metabolism. However, these effects are likely to be subtle compared with the issue faced by many female athletes of having an adequate energy intake or lack of dietary restraint to allow the theoretical targets for carbohydrate intake to be met. Many female athletes periodize their eating practices to cycle between phases in which energy considerations are prioritized and other situations in which the achievement of high carbohydrate availability is the key nutritional goal. Some guidelines for carbohydrate-rich food and meal and snack combinations can be made which would allow female athletes to meet requirements for other nutrients or nutritional goals for sport. Further research should also consider whether there are sex-based preferences for carbohydrate-rich food or food combinations that appeal to female athletes and thus contribute to food enjoyment and a healthy relationship with food.

REFERENCES

Achten, J., Halson, S. H., Moseley, L., Rayson, M. P., Casey, A., and Jeukendrup, A. E. 2004. Higher dietary carbohydrate content during intensified running training results in better maintenance of performance and mood state. *Journal of Applied Physiology* 96: 1331–1340.

Ahlborg, B., Bergstrom, J., Brohult, J., Ekelund, L. G., Hultman, E., and Maschio, G. 1967. Human muscle glycogen content and capacity for prolonged exercise after different diets. *Forsvarsmedicin* 3: 85–99.

American College of Sports Medicine, Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., and Stachenfeld, N. S. 2007. American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and Science in Sports and Exercise* 39: 377–390.

Andrews, J. L., Sedlock, D. A., Flynn, M. G., Navalta, J. W., and Hongguang, J. 2003. Carbohydrate loading and supplementation in endurance-trained women runners. *Journal of Applied Physiology* 95: 584–590.

Bemben, D. A., Boileau, T. A., Bahr, J. M., et al. 1992. Effects of oral contraceptives on hormonal and metabolic responses during exercise. *Medicine and Science in Sports and Exercise* 24: 434–441.

Berardi, J. M., Price, T. B., Noreen, E. E., and Lemon, P. W. 2006. Postexercise muscle glycogen recovery enhanced with a carbohydrate–protein supplement. *Medicine and Science in Sports and Exercise* 38: 1106–1113.

Bergstrom, J., Hermansen, L., Hultman, E., and Saltin, B. 1967. Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica* 71: 140–150.

Betts, J. A., and Williams, C. 2010. Short-term recovery from prolonged exercise: Exploring the potential for protein ingestion to accentuate the benefits of carbohydrate supplements. *Sports Medicine* 40: 941–959.

Betts, J. A., Williams, C., Boobis, L., and Tsintzas, K. 2008. Increased carbohydrate oxidation after ingesting carbohydrate with added protein. *Medicine and Science in Sports and Exercise* 40: 903–912.

Burke, L. M. 2010. Fueling strategies to optimize performance: Training high or training low? *Scandinavian Journal of Medicine and Science in Sports* 20 (Suppl. 2): 11–21.

Burke, L. M., Collier, G. R., Broad, E. M., Davis, P. G., Martin, D. T., Sanigorski, A. J., and Hargreaves, M. 2003. The effect of alcohol intake on muscle glycogen storage following prolonged exercise. *Journal of Applied Physiology* 95: 983–990.

Burke, L. M., Collier, G. R., Davis, P. G., Fricker, P. A., Sanigorski, A. J., and Hargreaves, M. 1996. Muscle glycogen storage after prolonged exercise: Effect of the frequency of carbohydrate feedings. *American Journal of Clinical Nutrition* 64: 115–119.

Burke, L. M., Collier, G. R., and Hargreaves, M. 1993. Muscle glycogen storage after prolonged exercise: The effect of the glycemic index of carbohydrate feedings. *Journal of Applied Physiology* 75: 1019–1023.

Burke, L. M., Cox, G. R., Cummings, N. K., and Desbrow, B. 2001. Guidelines for daily CHO intake: Do athletes achieve them? *Sports Medicine* 31(4): 267–299.

Burke, L. M., Hawley, J. A., Wong, S., and Jeukendrup, A. E. 2011. Carbohydrates for training and competition. *Journal of Sports Sciences* 29 (Suppl. 1): S17–S27.

Burke, L. M., Kiens, B., and Ivy, J. L. 2004. Carbohydrates and fat for training and recovery. *Journal of Sports Sciences* 22: 15–30.

Burke, L. M., Wood, C., Pyne, D. B., Telford, R. D., and Saunders, P. U. 2005. Effect of carbohydrate intake on half-marathon performance of well-trained runners. *International Journal of Sport Nutrition and Exercise Metabolism* 15: 573–589.

Campbell, S. E., Angus, D. J., and Febbraio, M. A. 2001. Glucose kinetics and exercise performance during phases of the menstrual cycle: Effect of glucose ingestion. *American Journal of Physiology* 281: E817–25.

Carter, J. M., Jeukendrup, A. E., and Jones, D. A. 2004a. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Medicine and Science in Sports and Exercise* 36: 2107–2111.

Carter, J. M., Jeukendrup, A. E., Mann, C. H., and Jones, D. A. 2004b. The effect of glucose infusion on glucose kinetics during a 1-h time trial. *Medicine and Science in Sports and Exercise* 36: 1543–1550.

Carter, S. L., McKenzie, S., Mourtzakis, M., Mahoney, D. J., and Tarnopolsky, M. A. 2001. Short-term 17β-estradiol decreases glucose R_a but not whole body metabolism during endurance exercise. *Journal of Applied Physiology* 90: 139–146.

Costill, D. L., Flynn, M. G., Kirwan, J. P., Houmard, J. A., Mitchell, J. B., Thomas, R. T., and Park, S. H. 1988. Effects of repeated days of intensified training on muscle glycogen and swimming performance. *Medicine and Science in Sports and Exercise* 20: 249–254.

Costill, D. L., Sherman, W. M., Fink, W. J., Maresh, C., Witten, M., and Miller, J. M. 1981. The role of dietary carbohydrates in muscle glycogen resynthesis after strenuous running. *American Journal of Clinical Nutrition* 34: 1831–1836.

Cox, G. R., Clark, S. A., Cox, A. J., Halson, S. L., Hargreaves, M., Hawley, J. A., Jeacocke, N., Snow, R. J., Yeo, W. K., and Burke, L. M. 2010. Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. *Journal of Applied Physiology* 109: 126–134.

Coyle, E. F. 2004. Fluid and fuel intake during exercise. *Journal of Sports Sciences* 22: 39–55.

Currell, K., and Jeukendrup, A. E. 2008. Superior endurance performance with ingestion of multiple transportable carbohydrates. *Medicine and Science in Sports and Exercise* 40: 275–281.

D'Eon, T. M., Sharoff, C., and Chipkin, S. R. 2002. Regulation of exercise carbohydrate metabolism by estrogen and progesterone in women. *American Journal of Physiology—Endocrinology and Metabolism* 283: E1046–E1055.

Devries, M. C., Mazen, J. H., Phillips, S. M., and Tarnopolsky, M. A. 2006. Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *American Journal of Physiology* 291: R1120–R1128.

Dolins, K. R., Boozer, C. N., Stoler, F., Bartels, M., DeMeersman, R., and Contento, I. 2003. Effect of variable carbohydrate intake on exercise performance in female endurance cyclists. *International Journal of Sport Nutrition and Exercise Metabolism* 13: 422–435.

Hackney, A. C. 1990. Effects of the menstrual cycle on resting muscle glycogen content. *Hormone and Metabolic Research* 22: 647.

Hackney, A. C. 1999. Influence of oestrogen on muscle glycogen utilization during exercise. *Acta Physiologica Scandinavica* 167: 273–274.

Hansen, A. K., Fischer, C. P., Plomgaard, P., Andersen, J. L., Saltin, B., and Pedersen, B. K. 2005. Skeletal muscle adaptation: Training twice every second day vs. training once daily. *Journal of Applied Physiology* 98: 93–99.

Hawley, J. A., and Burke, L. M. 2010. Carbohydrate availability and training adaptation: Effects on cell metabolism. *Exercise and Sport Sciences Review* 38: 152–160.

Hawley, J. A., Burke, L. M., Phillips, S. M., and Spriet, L. 2011. Nutritional modulation of training-induced skeletal muscle adaptation. *Journal of Applied Physiology* 110: 834–845.

Howarth, K. R., Moreau, N. A., Phillips, S. M., and Gibala, M. J. 2009. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *Journal of Applied Physiology* 106: 1394–1402.

Hulston, C. J., Venables, M. C., Mann, C. H., Martin, C., Philp, A., Baar, K., and Jeukendrup, A. E. 2010. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Medicine and Science in Sports and Exercise* 42: 2046–2055.

Ivy, J. L., Goforth, H. W., Damon, B. D., McCauley, T. R., Parsons, E. C., and Price, T. B. 2002. Early post-exercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *Journal of Applied Physiology* 93(4): 1337–1344.

Ivy, J. L., Katz, A. L., Cutler, C. L., Sherman, W. M., and Coyle, E. F. 1988. Muscle glycogen synthesis after exercise: Effect of time of carbohydrate ingestion. *Journal of Applied Physiology* 64: 1480–1485.

James, A. P., Lorraine, M., Cullen, D., Goodman, C., Dawson, B., Palmer, T. N., and Fournier, P. A. 2001. Muscle glycogen supercompensation: Absence of a gender-related difference. *European Journal of Applied Physiology* 85(6): 553–558.

Jentjens, R. L., Moseley, L., Waring, R. H., Harding, L. K., and Jeukendrup, A. E. 2004. Oxidation of combined ingestion of glucose and fructose during exercise. *Journal of Applied Physiology* 96: 1277–1284.

Jentjens, R. L., Underwood, K., Achten, J., Currell, K., Mann, C. H., and Jeukendrup, A. E. 2006. Exogenous carbohydrate oxidation rates are elevated after combined ingestion of glucose and fructose during exercise in the heat. *Journal of Applied Physiology* 100: 807–816.

Jentjens, R. L., van Loon, L. J. C., Mann, C. H., Wagenmakers, A. J. M., and Jeukendrup, A. E. 2001. Addition of protein and amino acids to carbohydrates does not enhance postexercise muscle glycogen synthesis. *Journal of Applied Physiology* 91: 839–846.

Jeukendrup, A. E. 2010. Carbohydrate and exercise performance: The role of multiple transportable carbohydrates. *Current Opinion in Clinical Nutrition and Metabolic Care* 13: 452–457.

Jeukendrup, A., Brouns, F., Wagenmakers, A. J. M., and Saris, W. H. M. 1997. Carbohydrate-electrolyte feedings improve 1 h time trial cycling performance. *International Journal of Sports Medicine* 18(2): 125–129.

Jeukendrup, A. E., and Chambers, E. S. 2010. Oral carbohydrate sensing and exercise performance. *Current Opinion in Clinical Nutrition and Metabolic Care* 13: 447–451.

Karelis, A. D., Smith, J. W., Passe, D. H., and Péronnet, F. 2010. Carbohydrate administration and exercise performance: What are the potential mechanisms involved? *Sports Medicine* 40: 747–763.

Keizer, H. A., Kuipers, H., Van Kranenburg, G., and Guerten, P. 1986. Influence of liquid and solid meals on muscle glycogen resynthesis, plasma fuel hormone response, and maximal physical working capacity. *International Journal of Sports Medicine* 8: 99–104.

Kern, M., Helsin, C. J., and Bezende, R. S. 2007. Metabolic and performance effects of raisins versus sports gel as pre-exercise feedings in cyclists. *Journal of Strength and Conditioning Research* 21(4):1204–1207.

Kimber, N. E., Ross, J. J., Mason, S. L., and Speedy, D. B. 2002. Energy balance during an Ironman triathlon in male and female triathletes. *International Journal of Sport Nutrition and Exercise Metabolism* 12: 47–62.

Kreider, R. B., Hill, D., Horton, G., Downes, M., Smith, S., and Anders, B. 1995. Effects of carbohydrate supplementation during intense training on dietary patterns, psychological status, and performance. *International Journal of Sport Nutrition* 5(2): 125–135.

McLay, R. T., Thomson, C. D., Williams, S. M., and Rehrer, N. J. 2007. Carbohydrate loading and female endurance athletes: Effect of menstrual-cycle phase. *International Journal of Sport Nutrition and Exercise Metabolism* 17(2): 189–205.

Nicklas, B. J., Hackney, A. C., and Sharp, R. L. 1989. The menstrual cycle and exercise: Performance, muscle glycogen, and substrate responses. *International Journal of Sports Medicine* 10: 264–269.

O'Keeffe, K. A., Keith, R. E., Wilson, G. D., and Blessing, D. L. 1989. Dietary carbohydrate intake and endurance exercise performance of trained female cyclists. *Nutrition Research* 9: 819–830.

Oosthuyse, T., and Bosch, A. N. 2010. The effect of the menstrual cycle on exercise metabolism: Implications for exercise performance in eumenorrhoeic women. *Sports Medicine* 40(3): 207–227.

Parkin, J. A. M., Carey, M. F., Martin, I. K., Stojanovska, L., and Febbraio, M. A. 1997. Muscle glycogen storage following prolonged exercise: Effect of timing of ingestion of high glycemic index food. *Medicine and Science in Sports and Exercise* 29: 220–224.

Pedersen, D. J., Lessard, S. J., Coffey, V. G., Churchley, E. G., Wootton, A. M., Ng, T., Watt, M. J., and Hawley, J. A. 2008. High rates of muscle glycogen resynthesis after exhaustive exercise when carbohydrate is coingested with caffeine. *Journal of Applied Physiology* 105: 7–13.

Piehl Aulin, K., Soderlund, K., and Hultman, E. 2000. Muscle glycogen resynthesis rate in humans after supplementation of drinks containing carbohydrates with low and high molecular masses. *European Journal of Applied Physiology* 81: 346–351.

Robinson, T. M., Sewell, D. A., Hultman, E., and Greenhaff, P. L. 1999. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. *Journal of Applied Physiology* 87: 598–604.

Ruby, B. C., Robergs, R. A., Waters, D. L., Burge, M., Mermier, C., and Stolarczyk, L. 1997. Effects of estradiol on substrate turnover during exercise in amenorrheic females. *Medicine and Science in Sports and Exercise* 29(9): 1160–1169.

Saris, W. H. M., Van Erp-Baart, M. A., Brouns, F., Westerterp, K. R., and Ten Hoor, F. 1989. Study on food intake and energy expenditure during extreme sustained exercise: The Tour de France. *International Journal of Sports Medicine* 10: S26–S31.

Sherman, W. M., Doyle, J. A., Lamb, D. R., and Strauss, R. H. 1993. Dietary carbohydrate, muscle glycogen, and exercise performance during 7 d of training. *American Journal of Clinical Nutrition* 57: 27–31.

Simonsen, J. C., Sherman, W. M., Lamb, D. R., Dernbach, A. R., Doyle, J. A., and Strauss, R. 1991. Dietary carbohydrate, muscle glycogen, and power output during rowing training. *Journal of Applied Physiology* 70: 1500–1505.

Smith, J. W., Zachwieja, J. J., Horswill, C. A., Pascoe, D. D., Passe, D., and Ruby, B. C. 2010a. Evidence of a carbohydrate dose and prolonged exercise performance relationship. *Medicine and Science in Sports and Exercise* 42(5): 84.

Smith, J. W., Zachwieja, J. J., Horswill, C. A., Pascoe, D. D., Passe, D., Ruby, B. C., and Pascoe, D. D. 2010b. Fuel selection and cycling endurance performance with ingestion of [13C]glucose: Evidence for a carbohydrate dose response. *Journal of Applied Physiology* 108: 1520–1529.

Suh, S., Casazza, G. A., Horning, M. A., Miller, B. F., and Brooks, G. A. 2003. Effects of oral contraceptives on glucose flux and substrate oxidation rates during rest and exercise. *Journal of Applied Physiology* 94: 285–294.

Tarnopolsky, M. A., Atkinson, S. A., Phillips, S. M., and MacDougall, J. D. 1995. Carbohydrate loading and metabolism during exercise in men and women. *Journal of Applied Physiology* 78(4): 1360–1368.

Tarnopolsky, M. A., Bosman, M., Macdonald, J. R., Vanderputte, D., Martin, J., and Roy, B. D. 1997. Postexercise protein-carbohydrate and carbohydrate supplements increase muscle glycogen in men and women. *Journal of Applied Physiology* 83(6): 1877–1883.

Tarnopolsky, M. A., Zawada, C., Richmond, L. B., Carter, S., Shearer, J., Graham, T., and Phillips, S. M. 2001. Gender differences in carbohydrate loading are related to energy intake. *Journal of Applied Physiology* 91: 225–230.

Triplett, D., Doyle, J. A., Rupp, J. C., and Benardot, D. 2010. An isocaloric glucose-fructose beverage's effect on simulated 100-km cycling performance compared with a glucose-only beverage. *International Journal of Sport Nutrition and Exercise Metabolism* 20: 122–131.

van Hall, G., Shirreffs, S. M., and Calbet, J. A. 2000. Muscle glycogen resynthesis during recovery from cycle exercise: No effect of additional protein ingestion. *Journal of Applied Physiology* 88: 1631–1636.

van Loon, L. J. C., Murphy, R., Oosterlaar, A. M., Cameron-Smith, D., Hargreaves, M., Wagenmakers, A. J. M., and Snow, R. 2004. Creatine supplementation increases glycogen storage but not GLUT-4 expression in human skeletal muscle. *Clinical Science* 106: 99–106.

van Loon, L. J. C., Saris, W. H. M., Kruijshoop, M., and Wagenmakers, A. J. M. 2000. Maximizing postexercise muscle glycogen synthesis: Carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *American Journal of Clinical Nutrition* 72: 106–111.

Walker, J. L, Heigenhauser, G. J. F., Hultman, E., and Spriet, L. L. 2000. Dietary carbohydrate, muscle glycogen content, and endurance performance in well-trained women. *Journal of Applied Physiology* 88: 2151–2158.

Wallis, G. A., Dawson, R., Achten, J., Webber, J., and Jeukendrup, A. E. 2006. Metabolic response to carbohydrate ingestion during exercise in males and females. *American Journal of Physiology—Endocrinology and Metabolism* 290(4): E708–E715.

Wallis, G. A., Yeo, S. E., Blannin, A. K., and Jeukendrup, A. E. 2007. Dose-response effects of ingested carbohydrate on exercise metabolism in women. *Medicine and Science in Sports and Exercise* 39(1): 131–138.

Yeo, W. K., Paton, C. D., Garnham, A. P., Burke, L. M., Carey, A. L., and Hawley, J. A. 2008. Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *Journal of Applied Physiology* 105: 1462–1470.

Zawadzki, K. M., Yaspelkis, B. B., and Ivy, J. L. 1992. Carbohydrate–protein complex increases the rate of muscle glycogen storage after exercise. *Journal of Applied Physiology* 72: 1854–1859.

Zderic, T. W., Coggan, A. R., and Ruby, B. C. 2001. Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *Journal of Applied Physiology* 90: 447–453.

3 Protein Requirements for the Female Athlete

Nancy R. Rodriguez

CONTENTS

INTRODUCTION

Dietary protein is a critical and often overlooked component of the female athlete's diet. An important and dynamic relationship exists between protein intake, energy balance, and the efficient use of dietary protein by the body in response to habitual exercise training. Adequate energy intake will "spare" protein for use in its myriad of important bodily functions (e.g., tissue growth, maintenance and repair, immune function, etc.); conversely, inadequate energy intake will necessitate that

protein be "sacrificed" for calories and, thus, the functions that rely on protein will be compromised.

Research suggests that protein utilization during exercise and therefore protein needs of women differ from their male counterparts; however, the magnitude of these differences and the implications regarding protein requirements remain somewhat controversial. This chapter will provide an overview of protein metabolism and highlight challenges and concerns regarding research designed to examine protein utilization in female athletes. Current recommendations regarding dietary protein intake and the practical application of this information to the lifestyles of healthy, competitive female athletes will also be presented.

PROTEIN AS A MACRONUTRIENT

Protein serves a number of structural and functional roles in the body. Structurally, protein is required for the synthesis of muscle tissue, tendons, ligaments, and even bone. Functionally, protein is involved in nutrient transport, endocrine control, immunity, and metabolic regulation. While protein can be used as fuel (i.e., ATP) to support muscular work, as will be described in more detail later in this chapter, using protein for energy compromises its structural and functional roles.

Protein is composed of amino acids, some of which are indispensable (essential) as they cannot be produced by the body and, thus, must be consumed as part of the diet (Table 3.1). Other amino acids are designated as dispensable (nonessential) because, given adequate nitrogen, they can be synthesized by the body from intermediates

TABLE 3.1
Indispensable, Dispensable, and Conditionally Essential Amino Acids

Indispensable Amino Acids	Dispensable Amino Acids	Conditionally Essential Amino Acids
Isoleucine*	Alanine	Arginine
Leucine*	Arginine	Cysteine
Lysine	Asparagine	Glutamine
Methionine	Aspartate	Histidine
Phenylalanine	Cysteine	Tyrosine
Tryptophan	Cystine	
Threonine	Glutamine	
Valine*	Glutamate	
	Glycine	
	Histidine	
	Proline	
	Serine	
	Taurine	
	Tyrosine	

* Branched-chain amino acids.

of metabolism or other amino acids. Finally, some amino acids are considered conditionally essential, meaning that they are normally dispensable but, under certain circumstances (e.g., severe stress or trauma, liver or kidney dysfunction, inborn errors of metabolism) they can become indispensable (Laidlaw and Kopple 1987).

THE CONCEPT OF PROTEIN TURNOVER

As a concept, protein turnover refers to the dynamic exchange between free amino acid pools and proteins in the body; from a functional standpoint it represents the process of protein synthesis relative to protein degradation. The difference between protein synthesis and protein breakdown reflects net protein balance. Figure 3.1 illustrates the basic concept of protein turnover including protein utilization by the body and the role of dietary protein in the provision of amino acids to the free amino acid pools that exist. Later in this chapter, the effects of exercise on protein turnover will be explored.

A dynamic state exists between protein synthesis and degradation with specific regard for amino acid availability. Inadequate dietary intake of amino acids or excessive utilization (oxidation) of amino acids during exercise will compromise the availability of amino acids for protein synthesis (and increase urinary nitrogen excretion). These relationships will be considered throughout the chapter with regard to recommended protein intakes for female athletes in the context of the effects of exercise on protein utilization.

Amino Acid Availability and Protein Synthesis

The scientific literature is replete with evidence that the provision of dietary amino acids can stimulate protein synthesis. In the fed state, when protein has been consumed and amino acids are abundant, protein synthesis exceeds protein breakdown

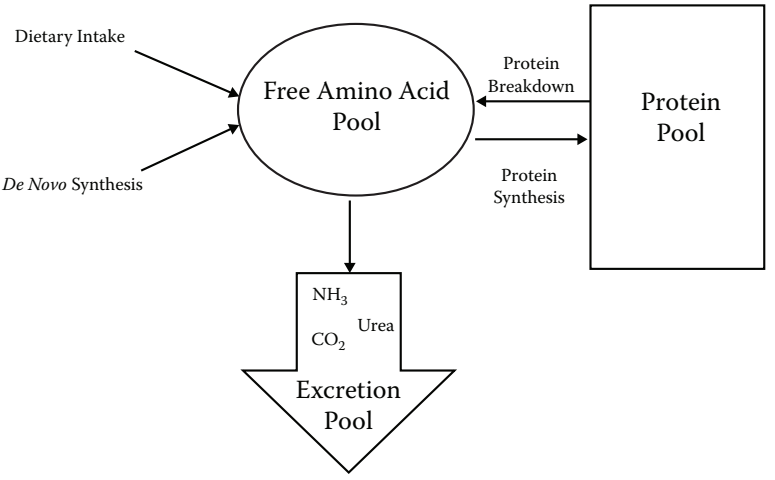


FIGURE 3.1 Protein turnover.

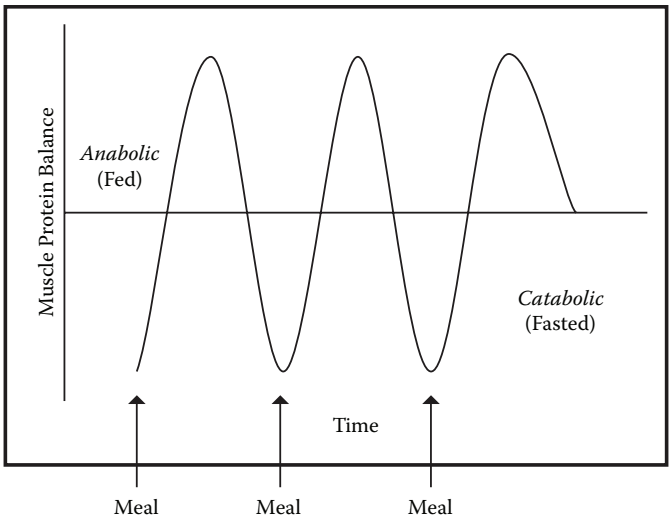


FIGURE 3.2 Skeletal muscle protein turnover.

such that protein balance becomes positive, an anabolic state exists, and lean body mass gains can be achieved (Biolo et al. 1997; Phillips 2004; Phillips et al. 2011). Conversely, in the fasted state, body proteins are degraded in order to support the free amino acid pools (Phillips et al. 1997; Kim et al. 2005). In this situation, protein breakdown exceeds protein synthesis resulting in a negative protein balance and a catabolic state. This concept of protein balance in the context of the fed and fasted state is illustrated in Figure 3.2.

In summary, consuming adequate dietary protein will maintain the amino acid pool and ensure amino acid availability for protein synthesis, thereby encouraging a positive net protein balance.

Regulation of Protein Synthesis

As indicated above, amino acid availability (as would occur in the fed state) is a strong stimulus for protein synthesis. Exercise has also been shown to be a potent stimulus of protein synthesis (Phillips et al. 2002; Rodriguez et al. 2007). The protein synthetic response in muscle varies with the type of exercise, the time of measurement, and the presence or absence of indispensible amino acids. Even though the physiological and biochemical processes underlying these adaptations are well understood, mechanisms specific to the regulation of protein synthesis continue to be characterized. The effects of exercise on protein turnover will be discussed at length later in this chapter.

In addition to amino acid availability and exercise, research suggests that energy availability, the hormonal environment, and initiation factors contribute to the regulation of protein synthesis (Anthony et al. 2000; Kimball 2007).

Energy availability is an important regulator of protein synthesis given that anabolism is energy dependent (Bell et al. 2005). At the cellular level, energy is required

for amino acid activation for protein assembly. At the dietary level, energy must be sufficient so that amino acids can be directed toward protein synthesis and not be used as an energy source (Fujita et al. 2007). In short, for protein synthesis to be optimized in female athletes, calorie needs must be met in addition to consuming sufficient amounts of dietary protein.

The hormone insulin is notable in stimulating protein synthesis (Kimball et al. 2002). Insulin is an anabolic hormone in the context of protein metabolism as it stimulates amino acid uptake into muscles, as well as enhances protein synthetic rates. Further, insulin has a role in the activation of a variety of initiation factors, or intracellular signaling proteins, central protein translation, and ultimately protein synthesis. Other hormones that have been implicated in increasing protein synthesis include IGF-1 and growth hormone (Bush et al. 2003). However, of these, the hormone insulin remains the most significant in supporting anabolic reactions specific to protein synthesis. Several nutritional interventions have repeatedly been shown to positively influence several of the points of intracellular regulation of protein synthesis in combination with or independent of the hormone insulin (Kimball 2007; Phillips 2008). The provision of intact proteins, amino acid mixtures, and the singular amino acid leucine alone or in combination has been shown to stimulate rates of protein synthesis by affecting one or several of these intracellular signaling proteins via, or independent of, the insulin dependent pathway (Anthony et al. 2000, 2001; Kimball et al. 2002; Koopman et al. 2005; Moore et al. 2009).

Regulation of Protein Breakdown

The process of protein catabolism is also highly regulated and essentially irreversible once proteins have been targeted for breakdown. Energy, protein availability, and to a certain extent the hormonal environment contribute to regulation of protein breakdown (Lee et al. 2004). In catabolic states, such as negative energy balance, protein breakdown increases in order to provide amino acids as gluconeogenic precursors for cellular energy production. Endogenous proteins, skeletal muscle in particular, are a primary site of proteolysis to maintain a supply of essential amino acids for the amino acid pool during the fasted state (Wolfe 2006). The process of atrophy, or muscle loss, involves a distinct increase in protein degradative processes (Du et al. 2004; Du and Mitch 2005; Lecker et al. 2006). The hormone cortisol has a central role in some of the processes specific to skeletal muscle protein breakdown (Du and Mitch 2005; Lecker et al. 2006).

Research has recently documented changes in enzymes and intracellular signals specific to protein synthetic and degradative pathways in endurance-trained men and women in response to variations in energy balance (Pasiakos et al. 2010; Carbone et al. 2012). Skeletal muscle protein synthesis was decreased and the activity of associated intracellular signaling proteins was reduced while the activity of signaling proteins specific to protein degradation was increased in response during a state of negative energy balance. These protein-specific metabolic responses were similar for men and women. The lack of sex-based differences in this study could be due to the small number of individuals (11 men and 7 women) who participated (Pasiakos et al. 2010). The scarcity of additional published scientific investigations that have

evaluated differences in protein utilization between male and female athletes subsequent to well-controlled diet and exercise interventions elicits the potentially flawed assumption that gender-specific responses are unlikely. As a result, recommendations regarding protein intakes remain similar for active men and women.

CHARACTERIZATION OF PROTEIN TURNOVER: NITROGEN BALANCE

Nitrogen balance is the oldest and, until recent years, was the most common method for examining protein utilization at rest and during exercise. Nitrogen balance is determined by subtracting nitrogen lost from the body in the urine and in the feces from nitrogen consumed in the diet as a component of dietary protein. Individuals with a positive nitrogen balance ($Nitrogen_{in} > Nitrogen_{out}$) are considered to be in an anabolic state. When the reverse is observed ($Nitrogen_{in} < Nitrogen_{out}$), a catabolic state usually exists.

There are a number of limitations to the nitrogen balance method, particularly as it pertains to the measurement of protein utilization. First, nitrogen balance is not static. A variety of nutrition parameters affect how the body uses protein and, therefore, impacts nitrogen balance measures including dietary protein and energy intake. For example, the body adapts to lower protein intakes by conserving nitrogen (i.e., decreasing the amount of nitrogen excreted). Conversely, excessive protein intake will transiently increase protein turnover and may result in an artifactual increase in nitrogen balance. Energy intake is a critical consideration because insufficient calorie intake may result in the use of protein as a fuel source. When protein intake is high, calorie intake low, and weight loss ensues, a negative nitrogen balance may not reflect losses of body protein. Rather, it may simply reflect the body's use of protein as an energy source.

Nitrogen balance studies do not provide any insight regarding specific aspects of protein synthesis or breakdown in general in the body or with respect to a particular organ or tissue. For example, nitrogen balance may reflect equilibrium ($Nitrogen_{in} = Nitrogen_{out}$) or maintenance, while muscle catabolism could be occurring as nitrogen is utilized for other functions in the body. The current Recommended Dietary Allowance (RDA) for protein for all healthy adults is based on nitrogen balance studies and has been widely criticized as being too low for certain populations such as active individuals and competitive athletes. This will be explored in more detail later in this chapter.

CHARACTERIZATION OF PROTEIN TURNOVER: STABLE ISOTOPE TECHNIQUES

The use of stable isotope methodology has provided insight regarding protein turnover in the body, as well as in specific tissues such as muscle, that extends beyond nitrogen balance. Not only can protein synthesis and protein breakdown in the body and in specific tissues, such as muscle (Figure 3.3), be better characterized with stable isotope techniques, but the use of protein as an energy source can also be considered.

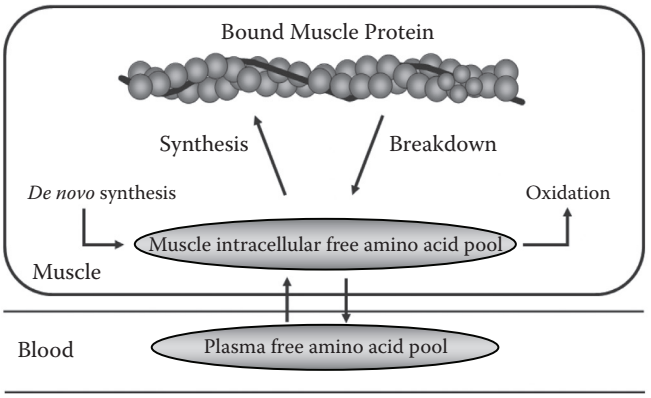


FIGURE 3.3 Concept of protein balance with fasting and feeding.

In brief, stable isotopes of amino acids can be used to follow or "trace" the metabolism of a particular amino acid of interest. Nonetheless, certain assumptions must be made for tracer methodology to be valid when using isotopically labeled amino acids (Wolfe and Chinkes 2004). The two most common are that the labeled amino acid, or tracer, behaves exactly the same as the respective unlabeled amino acid and that the tracer does not affect the metabolism of the tracee. As a result, an amino acid can be "labeled" on a constituent carbon, hydrogen, or nitrogen molecule and its metabolism can then be characterized. Of course, therein lies a limitation of such techniques as it is assumed that the metabolism or utilization of a particular amino acid is representative of other amino acids. However, meticulous scientific effort has been taken to select amino acid tracers for which reasonable and valid assumptions can be made for the purpose of characterizing protein turnover in the body and in various body organs of healthy, active men and women (Wolfe and Chinkes 2004). Findings from these types of investigations provide a foundation for much of the following discussion.

EFFECTS OF EXERCISE ON PROTEIN TURNOVER

While exercise, in general, increases protein turnover, the specific effects on protein synthesis and catabolism and, thus, net protein balance, are dependent upon the type, intensity, and duration of the exercise as well as the nutritional status of the athlete as she enters and engages in the exercise bout. Research also suggests there may be a differential effect of sex on protein turnover during exercise (Burd et al. 2009). Each of these factors will be considered below.

Exercise Type: Resistance versus Endurance Exercise

In order for muscle to increase in size, the production of new muscle proteins must increase and muscle protein synthesis must be greater than breakdown such that protein balance is positive. A single session of resistance training stimulates an increase

in both muscle protein synthesis and breakdown in the period following the exercise; however, the degree of synthesis exceeds breakdown such that the net balance is positive. The increase in synthesis appears to be more pronounced in untrained subjects, where elevations in synthesis can persist upward to 48 hours post-exercise (Phillips et al. 1999; Hartman et al. 2006). Routine strength training has been shown to lead to an attenuated response that is believed to be due to a more efficient use of dietary protein by trained individuals compared to those individuals in the initial stages of a strength training program (Phillips et al. 2002; Phillips 2004). Whether an individual is naïve to resistance exercise or experienced with this mode of training, consumption of a protein-sufficient diet in combination with adequate calories will result in a positive net protein balance. These observations in men are usually extended to women even though there is little evidence on which to base this practice.

As mentioned previously, in the fasted state, net muscle protein balance is negative (i.e., breakdown is greater than synthesis). Even though resistance exercise attenuates the catabolic nature of fasting by leading to a less negative net balance, net muscle protein balance will remain negative if food is not provided after training due to the fact that increases in rates of breakdown occur simultaneously with elevated rates of synthesis. Simply put, in order for muscles to experience hypertrophy, it is critical that amino acids, as well as sufficient energy, are available at the same time the stimulus for increased synthesis is present (Biolo et al. 1997; Tipton and Wolfe 2001). Resistance exercise and amino acids work together to stimulate muscle growth (see Figure 3.4) (Burd et al. 2009).

During endurance exercise, energy is needed by the muscles for muscular work. Because protein synthesis is an anabolic process that, by definition, requires energy, muscle protein synthetic rates are reduced or unchanged during endurance exercise not only because energy is being used for fuel instead, but because a small amount of amino acids are being oxidized for fuel. It is estimated that under normal circumstances, protein oxidation accounts for approximately 2 to 5% of the substrates utilized during endurance exercise (Lemon and Nagle 1981; Carraro et al. 1990). Because endurance athletes do not experience significant muscle atrophy, the catabolic phase during exercise must be balanced by an anabolic recovery state.

Researchers have observed that protein synthesis following endurance exercise is increased above that noted at rest or before exercise (Carraro et al. 1990). The increase in protein turnover noted in response to an acute bout of endurance exercise

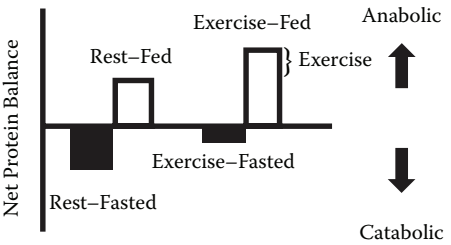


FIGURE 3.4 Effects of feeding and exercise on protein balance.

persists with endurance training (Pikosky et al. 2006). McKenzie et al. (2000) found that endurance training resulted in a decrease in leucine oxidation during exercise and an increase in protein synthesis post-exercise, a condition that could lead to improved protein balance over time if protein and total energy intake are adequate during training. Similarly, Pikosky et al. (2006) evaluated the effect of 6 weeks of aerobic exercise training on whole-body protein turnover in healthy men and women and noted increases in protein breakdown and protein synthesis and a decrease in leucine oxidation compared to the untrained state. No differences in the whole-body protein turnover response or skeletal muscle protein synthesis were noted between men and women (Pikosky et al. 2006).

The above data suggest that while endurance exercise suppresses protein synthesis and stimulates protein degradation *during* the exercise session, there is an increase in protein synthesis and net positive protein balance in the post-exercise period. Moreover, endurance training upregulates whole-body protein turnover, but there is an eventual conservation of protein noted with training. Research has also shown that the provision of exogenous amino acids can attenuate the rate of protein breakdown during endurance exercise (Miller et al. 2007). Further, increasing habitual protein intake also modulates protein utilization by the muscle in support of protein synthesis for endurance athletes (Bolster et al. 2005).

Exercise Intensity

Exercise intensity is inversely related to oxygen availability. That is, as exercise intensity increases, oxygen availability to the muscle is reduced. As a result, the ability to oxidize fat is reduced and the muscle relies to a greater extent on anaerobic substrate (i.e., creatine phosphate, glucose, and amino acids) metabolism for energy. Because of the rather limited creatine and glycogen stores, intense exercise increases the likelihood that protein will be broken down to its constituent amino acids to provide energy for muscular work (Lemon and Nagle 1981).

Research indicates that when low- to moderate-intensity exercise (≤50% VO_{2max}) is performed, zero nitrogen balance can be attained at protein intakes at or near the RDA (i.e., 0.8 g/kg/d). However, as the intensity of exercise training increases, greater protein intakes may be required for the achievement of nitrogen balance (Todd et al. 1984). Studies of moderate-intensity exercise (≥50% VO_{2max}) have found that ~1 $g^{-1} \cdot day^{-1}$ is not adequate for achieving nitrogen balance. Furthermore, in elite endurance athletes undergoing intense training, the level of protein required for nitrogen balance is estimated to be in the range of 1.5 to 1.8 $g^{-1} \cdot day^{-1}$ (Lemon 1998).

Exercise Duration

During exercise of moderate intensity, when oxygen is readily available, both fatty acids (derived from intramuscular triglycerides and peripheral adipose stores) and glucose (primarily from muscle glycogen) are oxidized. As the duration of exercise increases and glycogen stores become depleted, there is a greater reliance on protein as a fuel source (Carraro et al. 1990). Thus, glycogen availability directly affects the oxidation of protein during endurance exercise (Howarth et al., 2010). Inadequate

glycogen stores at the beginning of an endurance bout results in a greater utilization of amino acids for energy (Lemon 2000). Similarly, failure to consume exogenous glucose during exercise will cause an increase in amino acid oxidation (Lemon 2000). These observations provide additional support for the importance of consuming adequate carbohydrate before, during, and after prolonged, moderately intense exercise. Not only will adequate carbohydrate intake optimize athletic performance, but it will minimize protein catabolism in the endurance athlete (Cermak et al. 2009).

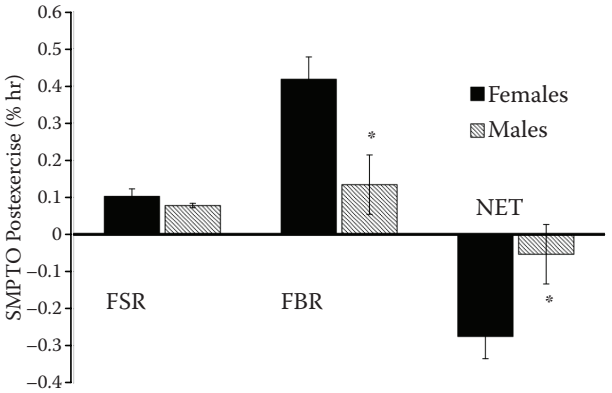
Sex Differences in Protein Turnover during Exercise

Research suggests that there are gender differences in protein metabolism during exercise, although the precise nature and extent of these differences as well as the implications with respect to gender-specific protein recommendations are not completely understood.

Mark Tarnopolsky and colleagues have contributed the majority of scientific investigations examining gender differences in substrate utilization during exercise (see Chapter 1) with some studies showing that males oxidize more protein than females during submaximal exercise of the same relative intensity (Tarnopolsky et al. 1990). Of significance to this chapter is a study that examined substrate oxidation in similarly trained males and females during a 15.5 km run (~65% VO_{2max}) and found that urinary urea nitrogen excretion was greater in males suggesting a greater protein utilization. Other researchers have conducted similar studies utilizing similar exercise protocols with trained men and women and found higher rates of amino acid oxidation at rest and during endurance exercise in men, suggesting that women use protein to a lesser extent than their male counterparts during moderately intense exercise (Phillips et al. 1993; Lamont et al. 2001, 2003).Gender differences in skeletal muscle fiber type are likely an important consideration in protein metabolism. In response to exercise of the same type, intensity, and duration, females exhibit increased Type I fiber area while males show greater Type II fiber area. Type I fibers have a greater oxidative capacity and greater ability to utilize lipids for fuel, while Type II fibers are better able to utilize glycogen. Differences in fiber type composition between males and females may be one possible explanation for observed gender differences in protein utilization during exercise (see Chapter 1).

Given that males rely more on amino acids for fuel during endurance exercise and that gender differences in hormonal response to exercise have been documented, differences in the skeletal muscle protein turnover response to exercise seem likely between men and women. In a preliminary study designed to explore the influence of gender on skeletal muscle protein turnover response to an acute bout of endurance exercise, male and female runners who habitually consumed 1.8 g/kg/d of protein completed a 75 minute run at 70% VO_{2max} (Gaine 2005). The results indicated that males and females demonstrated similar rates of skeletal muscle protein synthetic rates during recovery; however, the females had greater protein breakdown rates and a more negative net protein balance than the males (Figure 3.5).

It is important to acknowledge that in this particular study the male and female subjects were studied in the fasted state. While this is a logical first step in determining



*Different from females, $p < 0.05$

FIGURE 3.5 Effects of gender on skeletal muscle protein turnover response to an acute bout of endurance exercise.

gender differences in response to exercise alone, there is a need to perform studies in which nutrients are consumed during and following exercise to determine if sex-based differences in response to exogenous substrate provision do exist. Further, differences in level of fitness between the men and women may have contributed to the observations. The male runners in this study were extremely fit, and the female runners, although endurance trained, were not matched to the men with regard to fitness level. Although a difficult task, males and females should be more similarly matched for level of training based on years of training and training volume in future studies.

Nonetheless, despite the study limitations, the preliminary observations lend further support to the existing data that gender differences in protein metabolism may exist in response to endurance exercise. Additional studies of these responses are needed before conclusive statements regarding the magnitude and direction of the differences in protein utilization between female and male athletes and recommendations for intake based on these differences can be made. Specifically, studies comparing gender differences in response to exercise in untrained individuals are important, because this is the period of time when many of the adaptations to exercise occur. Further, given that gender differences in substrate and endocrine changes during a prolonged recovery period following endurance exercise have been documented while variables known to confound these responses—diet prior to measurements, training volume, and menstrual phase in females—were controlled (Vislocky et al. 2008), studies investigating gender differences when females are in different phases of the menstrual cycle are needed.

These points become more relevant given that studies have shown that sex hormones influence protein metabolism (Ferrando et al. 1998; Toth et al. 2006a, 2006b; Tipton and Ferrando 2008). Of these, the predominantly male hormone testosterone is recognized as a potent anabolic stimulus for achieving a positive protein balance. Testosterone elicits this effect by enhancing the re-utilization of amino acids

derived from muscle protein degradation for muscle protein synthesis (Tipton and Ferrando 2008). In women, the effects of reproductive hormones, estrogen in particular, on protein utilization are less understood as scientific studies in this area are lacking. In healthy, young eumenorrheic women, Toth and colleagues noted changes in whole-body protein utilization that reflected reductions in protein synthesis and protein breakdown and suggested that ovarian hormones (i.e., estrogen) participate at some level in the regulation of protein turnover (Toth et al. 2006a). These researchers then examined the effect of menopausal status and hormone replacement therapy on protein metabolism and found no differences in protein metabolism between healthy premenopausal and postmenopausal women (Toth et al. 2006b). There were no effects of hormone replacement therapy on whole-body protein turnover. Although this work might be considered preliminary in nature, it is nonetheless intriguing and challenges the scientific community to delineate the role of reproductive hormones with regard to differential exercise-elicited responses in protein utilization between men and women in carefully designed investigations. Further elucidation of these differences in gender responses and the identification of possible mechanisms specific to women and men are needed so that the design of training programs and nutritional interventions specific to female endurance athletes can be explored.

RECOMMENDED PROTEIN INTAKES

In 2010 the Institute of Medicine published *Dietary Reference Intakes* (DRIs) for specific nutrients, including protein (2010 DRIs). The DRIs are a set of reference values that include the commonly recognized RDA, which is defined as "the average daily nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) of healthy individuals" (National Research Council 2005). While the RDA for protein for adults remains at 0.8 grams of protein per kilogram body weight, or approximately 0.4 grams of protein per pound, the DRIs for protein range from approximately 0.7 to 1.5 grams per kilogram (or about 0.3 to 0.7 grams per pound). The DRIs are based on the concept that there is a range of protein intakes for optimal protein utilization, which is referred to as the Acceptable Macronutrient Distribution Range (AMDR) for protein (National Research Council 2005). Having a range of protein intakes is useful in individualized diet design for the female athlete given variations in training programs and competitive seasons, which translates into variations in energy needs and macronutrient composition of the diet. While the most common definition for protein requirements calls for a minimum level of protein that will balance losses and maintain nitrogen equilibrium, an athlete in training seeks a level of protein intake that is "optimal," not simply "adequate." That is, a level of protein intake that promotes a positive net protein balance by enhancing protein synthesis and limiting protein breakdown while maintaining positive nitrogen balance.

The level of dietary protein intake influences protein turnover rates such that higher protein intakes increase the basal rate of both protein synthesis and protein degradation. Adaptation to a low-protein diet is associated with a reduction in protein turnover rate in young men and women, which results in a new steady state

after approximately 5 to 7 days on a lower-protein diet (Reeds and Garlick 1984). Although the body will adapt to a lower level of protein intake, the long-term impact of increases and decreases in whole-body protein turnover in female athletes is not known. Muscle is constantly remodeling as protein turnover is a dynamic event. The effect of routine exercise in combination with varied levels of habitual protein intakes is difficult to study and is an area of continued investigation. A well-controlled diet intervention study in trained male runners showed variations in habitual protein intake affected whole-body and skeletal muscle protein utilization to the extent that protein intakes approximating the RDA appeared insufficient for these male endurance athletes (Bolster et al. 2005). Whether this observation translates directly to recommendations for female athletes is not known. However, the recommendation that protein intakes of female athletes exceed the RDA remains impartial to gender.

Nitrogen balance studies have indicated that protein needs are greater in men engaging in chronic endurance and resistance exercise and generally exceed the RDA of 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ (Gontzea et al. 1975; Tarnopolsky 2004; Tipton and Witard 2007). Findings from studies that include men and women suggest that exercise training actually improves nitrogen utilization (i.e., conserves nitrogen) allowing nitrogen balance to be achieved at protein intakes of 0.8 $g \cdot kg^{-1} \cdot day^{-1}$ so long as energy intake is sufficient (Pikosky et al. 2006). Therein lies the contradiction specific to basing recommended protein intakes for female and male athletes on nitrogen balance data. Because nitrogen balance can be achieved with efficient recycling of amino acids and an overall decrease in protein turnover with lower protein intakes when energy balance exists (Pikosky et al. 2006; Gaine et al. 2006, 2007), it is generally accepted that nitrogen balance studies may underestimate optimal protein intakes in athletes with rates of protein turnover that exceed those of the general population. There have been no studies indicating that nitrogen balance does not become more positive when protein intakes are increased. Therefore, it is important to consider that for *optimal* adaptations to exercise training to occur, female athletes, as well as their male counterparts, will benefit from protein intakes greater than those of the non-exercising population.

The Joint Position Paper on Nutrition and Athletic Performance includes a recommendation for protein intakes that range from 1.2 to 1.7 $g^{-1} \cdot kg^{-1} \cdot day^{-1}$ and at least 12 to 15% of energy from protein for healthy athletes irrespective of sex (Rodriguez et al. 2009). While level of dietary protein intake influences whole-body and skeletal muscle protein utilization, habitual protein intake in excess of 1.8 $g^{-1} \cdot kg^{-1} \cdot day^{-1}$ appears to be of little benefit to the athlete (Bolster et al. 2005).

Recommendations Specific to Exercise Type

Because resistance training stimulates an increase in protein turnover and results in muscle damage, it is commonly recommended that individuals who resistance train consume protein at intakes above the RDA in order to support elevations in protein synthesis and aid in muscle repair. Analysis of several nitrogen balance studies indicates that a protein intake of ~1.3 $g^{-1} \cdot kg^{-1} \cdot day^{-1}$ protein per day is sufficient for strength-trained individuals to achieve nitrogen balance (Phillips and Van Loon

2011). It has been recommended that individuals engaging in resistance training programs aim for a protein intake of 1.4 to 1.7 g/kg/d (Rodriguez et al. 2009; Phillips and Van Loon 2011). For individuals just beginning a resistance training program, protein needs may initially be higher because of the heightened effect of resistance exercise on increasing skeletal muscle protein turnover in these persons (Phillips 2004).

The timing, composition, and quantity of amino acids needed in relation to resistance exercise to optimize the muscle protein synthetic response remains an area of interest and will be described later in this chapter. Because variation in protein synthetic response is insignificant when amino acids are consumed before or after a training bout, individuals have some flexibility with timing of protein intake (Tipton et al. 2001, 2007). The amount of amino acids needed to elicit this anabolic effect is approximately 6 to 8 g of essential amino acids, which equates to approximately 20 to 30 grams of intact protein (Moore et al. 2009; Breen and Phillips 2012). Whether this amount of protein, or more specifically essential amino acids, is consumed prior to or following the training bout is less important than actually assuring adequate essential amino acids are available for muscle protein repair once the exercise bout is complete (Tipton and Witard 2007). Quality and not quantity of protein should be considered because evidence shows that nonessential amino acids are not necessary to increase protein synthesis. Rather, essential amino acids are critical to maximize protein utilization in response to routine resistance exercise (Tipton et al. 1999). Because the majority of this work has been conducted in men, the recommendation is once again extended to female athletes without an equivalent amount of evidence available to support the recommendation.

Protein requirements for endurance athletes have not been as well studied as those for strength athletes. Indeed, protein has typically not been considered to play a major role in the diets of endurance athletes because carbohydrates and fat are the primary fuel sources during endurance exercise and because muscle hypertrophy is not typically a goal of endurance training (Rodriguez et al. 2007). Nonetheless, as was discussed earlier in the chapter, protein metabolism is affected by endurance exercise, especially when either energy intake or carbohydrate intake is inadequate and, thus, both the amount and timing of protein intake are important for endurance athletes.

There is an increased reliance on amino acid oxidation for energy to support muscular work as exercise of moderate intensity increases in duration (Lemon 1998). The extent of the increase in amino acid oxidation depends on the intensity and duration of exercise as well as the degree of glycogen depletion. Based largely on a limited number of nitrogen balance studies, it is recommended that endurance athletes (both males and females) aim for a protein intake of 1.2 to 1.4 $g^{-1} \cdot kg^{-1} \cdot day^{-1}$ while consuming carbohydrates at a level that will maintain or replenish glycogen stores (5 to 10 $g^{-1} \cdot kg^{-1} \cdot day^{-1}$) (Lemon and Nagle 1981; Rodriguez et al. 2009). Similar to strength training, evidence suggests that persons beginning an endurance exercise program may have increased protein needs during the initial few weeks (Gontzea et al. 1975). Consumption of calories sufficient to maintain energy balance concurrently with recommended intakes of protein and carbohydrate during this time will improve protein utilization in response to endurance training. This concept applies to competitive female, as well as male, athletes.

Protein Consumption during Exercise

A number of studies have demonstrated possible performance benefits when protein is consumed with carbohydrate (CHO) during endurance exercise (Koopman et al. 2005; Gibala 2007; Saunders et al. 2007). For example, Saunders et al. (2007) showed improvements in cycling performance and attenuation of muscle damage when protein was added to a standard 6% CHO solution or CHO gel. Similarly, Miller et al. (2007) provided runners with fat-free milk throughout a 1-hour endurance run and evaluated whole-body protein turnover during recovery. Protein breakdown and synthesis were decreased and leucine oxidation increased following the milk-supplemented run. The authors suggested that use of exogenous amino acids for fuel during the run likely spared glycogen during exercise, which is beneficial during longer, more intense exercise regimens. A major criticism of these studies, however, is that individuals consuming the same amount of calories were not included in the study design. As a result, the caloric difference between the treatment vehicles may have contributed to the difference noted between treatment groups. Studies that have used isoenergetic controls have found no significant endurance performance benefits when protein is added to a CHO solution consumed during endurance exercise (VanEssen and Gibala 2006; Valentine et al. 2008; Cermack et al. 2009), although it should be noted that no decrements in performance were evidenced either.

For strength and power athletes, on the other hand, ingesting protein plus CHO during prolonged resistance training (greater than ~45 min) may ameliorate muscle catabolism, preserve muscle glycogen, and enhance muscle protein (PRO) accretion (Kerksick et al. 2008). Although the mechanism by which CHO positively affects PRO status is not precisely known and is likely multifaceted, at least part of the effect is attributed to insulin that has been shown to be a potent inhibitor of muscle protein breakdown (MPB) in men following consumption of an amino acid, protein, and carbohydrate mixture after resistance exercise (Borsheim et al. 2004). Because insulin's role in the global regulation of protein synthesis is well established (Kimball et al. 2002; Kimball 2007), it is usually assumed that the protein synthetic response noted with a mixed macronutrient beverage after an exercise (Borsheim et al. 2004; Lunn et al. 2012) bout in men can be extended to women.

Protein Consumption Post-Exercise

Timing exogenous protein intake around exercise can impact amino acid availability in the free amino acid pool, potentially sparing muscle degradation and enhancing protein synthesis. Increasingly, studies have examined protein co-ingestion with carbohydrate post-exercise on performance and markers of muscle damage as well as whole-body protein turnover. As a result of these studies, protein has become a contemporary nutrient in support of recovery from endurance exercise (Rodriguez 2009).

Levenhagen et al. (2002) observed that only 10 g of protein and 8 g of carbohydrate after cycling for 60 minutes at moderate intensity (60% VO_2 peak) was enough to induce protein synthesis and increase whole-body protein turnover by 15%.

Koopman et al. (2005) examined the effects of carbohydrate (0.7 $g \cdot kg^{-1} \cdot hr^{-1}$ or ~709 g total) or CHO plus protein (CHOPr) (0.7 $g \cdot kg^{-1} \cdot hr^{-1}$ or ~709 g total CHO plus 0.25 $g \cdot kg^{-1} \cdot hr^{-1}$ protein or ~253 g protein over 14 hours) during rest, prolonged endurance exercise, and recovery in well-trained male athletes. Whole-body protein turnover was 50% higher in CHOPr trials compared with CHO. Protein synthesis decreased during exercise compared with rest or recovery phases with both supplement trials; however, protein breakdown was decreased in CHOPr compared with CHO. Protein oxidation was highest during exercise in both trials compared with rest or recovery phases; however, protein oxidation during all three phases was higher in CHOPr compared with CHO. Even in the presence of increased oxidation, decreased protein breakdown with CHOPr led to a net protein balance that was less negative during all three phases. The amount of protein given throughout the study was similar to habitual intake of runners in a study reported by Miller et al. (3.5 $g \cdot kg^{-1} \cdot d^{-1}$ versus 3.12 $g \cdot kg^{-1} \cdot d^{-1}$) (Miller et al. 2007), indicating the importance of protein timing, as well as amount of protein routinely consumed, on favorable NET protein balance.

A well-designed study using three recovery beverages following 2 hours of cycling at varying intensities in recreationally active men showed increased leucine oxidation and flux, decreased protein breakdown, and a positive net protein balance with CHOPr versus CHO alone. Beverages were provided every 15 minutes for 3 hours following recovery and consisted of either 1.2 $g \cdot kg^{-1} \cdot hr^{-1}$ CHO, 1.2 $g \cdot kg^{-1} \cdot hr^{-1}$ CHO plus 0.4 $g \cdot kg^{-1} \cdot hr^{-1}$ protein (matched for CHO and providing ~108 g total protein based on average body weight of 90 kg), or 1.6 $g \cdot kg^{-1} \cdot hr^{-1}$ CHO (matched for total calories of protein beverage).

Provision of high-quality protein following an endurance bout is also beneficial to whole-body and skeletal muscle protein utilization (Lunn et al. 2012). Consumption of a mixed carbohydrate and protein drink following an endurance exercise bout affected intracellular components of proteolytic pathways such that protein degradation was reduced in endurance-trained men (Lunn et al. 2012).

Rowlands and Wadsworth (2011) evaluated whether consumption of a high-protein (0.7 $g \cdot kg^{-1} \cdot hr^{-1}$) beverage during a 4-hour recovery period following a ride would provide any benefit to subsequent performance in well-trained female cyclists compared to that noted when a low-protein (0.1 $g \cdot kg^{-1} \cdot hr^{-1}$) drink was consumed. The results indicated no clear influence of protein quantity on subsequent performance in females. However, findings from nitrogen balance studies suggested that protein needs for these women were in excess of the current RDA and 0.65 times greater than their male counterparts (Rowlands and Wadsworth 2011). The majority of work in this area has been conducted in male athletes. The Rowlands and Wadsworth investigation (2011) is unique in its examination of the effects of post-exercise protein consumption on recovery in female athletes. Therefore, generalizations regarding protein needs of athletes irrespective of gender should be cautiously undertaken. This study (Rowlands and Wadsworth 2011), as well as others (Phillips et al. 1993; Lamont et al. 2001, 2003), indicate that additional research is warranted to elucidate whether protein recommendations for female endurance athletes should be distinguished from those of their male counterparts.

Similarly, research in resistance-trained athletes essentially reflects a male-specific population response. Because essentially no data exist from studies investigating post-exercise recovery nutrition and protein utilization in women following resistance exercise, recommendations for nutritional supplementation post-strength training in women are, in practice, the same as those for men. However, these recommendations will often highlight micronutrients important to health and performance for women (i.e., calcium and iron) (Hausswirth and Le Meur 2011).

PROTEIN INTAKES OF ACTIVE, YOUNG WOMEN

A number of studies have documented the habitual protein intakes of female athletes (Hinton et al. 2004). On average, female athletes were noted to consume approximately 1.5 $g^{-1} \cdot kg^{-1} \cdot day^{-1}$, which would seem to indicate that, on average, protein intakes of female athletes are more than adequate. Nonetheless, it is important to recognize that this value may be misleading. A mean value can easily be biased by one or two high intake values and thus may not reflect the lower intakes of a significant number of female athletes. Moreover, while average protein intakes appear to be adequate, these intake levels may not be sufficient in the context of low energy intake (which is common among female athletes). Research consistently shows that women who participate in sports typically associated with a "thin build" are at risk for consuming insufficient protein because of low calorie intakes (Hinton et al. 2004; Nattiv et al. 2007; Hausswirth and Le Meur 2011). Female athletes in general, and endurance athletes in particular, as well as female athletes engaging in weight loss practices, are also at risk for inadequate protein intake. For that reason, it is critical for female athletes to be cognizant of consuming adequate protein with sufficient energy to optimize muscle protein synthesis, muscle repair, and whole-body protein balance.

CONSIDERATIONS FOR PROTEIN SOURCE

Because our need for protein is dictated by our need for the essential amino acids, foods that provide larger amounts of essential amino acids are preferable when considering protein balance. Although most vegetables and grains contain all of the essential amino acids, foods such as meat, eggs, fish, and dairy products provide a greater total amount of essential amino acids on a gram-per-gram basis and often on a gram-per-calorie basis. Thus, for active women interested in weight maintenance, gains in lean body mass, or even weight loss, a diet containing adequate amounts of protein would require a greater consumption of cereals, vegetables, and grains to correspond with the essential amino acid content of a diet for which animal proteins were the primary dietary source of essential amino acids.

There is little information regarding protein source and protein utilization in the general population, let alone physically active women. Furthermore, most of the work on nutrient modulation of skeletal protein metabolism in combination with exercise protocols has utilized amino acid drinks (versus whole foods), focused on resistance exercise, or has been short term in design. A study done by Haub and

coworkers (2002) showed greater gains in fat-free mass and skeletal muscle mass with resistance training in older men consuming a meat-containing diet compared to those older men consuming a lacto-ovo vegetarian diet for which total calorie and protein intakes were the same. Although this research was conducted in older men, it suggests that protein source may possibly influence protein utilization and provides a rationale for considering protein source in diet design for female athletes.

Potentially, recommendations for optimal protein intake in physically active women might include guidelines for the source of protein because, as a population, female athletes often require fewer calories and typically participate in sports for which maintenance of a lean physique is often emphasized. Because the beneficial effects of amino acid administration are the same when either a complete mix of amino acids or just the essential amino acids alone are consumed (Tipton et al. 1999), it would follow that varied protein sources in the diet would provide the essential amino acids required to support protein synthesis and promote maintenance, repair, and growth of muscle mass. The branched-chain amino acid leucine is of particular significance to this discussion because this indispensable amino acid is a potent nutritional signal in support of skeletal muscle protein synthesis (Anthony et al. 2000, 2001; Kimball 2007). Dairy proteins, such as whey and casein, as well as other animal sources of protein contain more leucine than an equivalent amount of protein from vegetable sources. Discussions continue as to whether leucine provided in intact protein such as that found in milk or protein isolates (i.e., whey, casein) is preferable in eliciting a protein synthetic response equivalent to that noted with supplementation of leucine alone. From a nutritional perspective, it would be preferable for female athletes to consume a nutrient-dense source of protein from a whole food such as skim milk to obtain a variety of micronutrients, including calcium, vitamin D, and vitamin A, from a natural source (Phillips 2004, 2010).

CONSIDERATIONS FOR VEGETARIAN ATHLETES

As indicated previously, the amount of essential amino acids on a gram-for-gram basis is lower in plant foods compared to animal products. Moreover, the bioavailability of amino acids from plant-based foods appears to be lower than that of animal-based foods. Thus, female athletes who adhere to vegetarian diet plans need to include plant-based foods that are significant sources of protein in their routine diets. This is particularly true for those female athletes who follow a completely vegan diet (i.e., consume no animal products whatsoever). In addition, it is crucial that female athletes who practice vegetarianism consume sufficient total calories to meet the energy demands of their sport, so as to minimize protein used for energy (Larson-Meyer 2007). Vegetarian diets that are appropriately planned can provide adequate energy and the appropriate range of carbohydrate, protein, and fat to support performance and health (Venderley and Campbell 2006; Larson-Meyer 2007). Female athletes can meet their protein needs from a predominantly or solely plant-based menu when a variety of these foods are habitually consumed in an energy adequate diet. Table 3.2 contains examples of protein-rich, nutrient-dense foods that can easily be incorporated into a female athlete's diet while accommodating particular food preferences.

TABLE 3.2
Protein-Rich, Nutrient-Dense Foods for Female Athletes

Animal Sources	Plant Sources
2 eggs	1½ cup soy milk
1½ cup reduced-fat milk	¾ cup beans or lentils
1 oz reduced-fat cheese	4 oz tofu
1 cup low-fat fruit yogurt	2 cups cooked pasta
½ cup cottage cheese	3 cups cooked rice
1–2 oz lean beef	3 cups whole grain cereal
2 oz chicken	4 slices whole grain bread
2 oz grilled fish or packaged tuna/salmon	2 oz nuts

Note: Each serving provides ~10–15 grams of protein.

IMPORTANCE OF ENERGY BALANCE TO PROTEIN UTILIZATION IN AND PROTEIN RECOMMENDATIONS FOR ACTIVE WOMEN

Female athletes, like their nonathletic counterparts, are often concerned about body weight, and many female athletes report actively trying to lose weight to improve performance, appearance, or both (Nattiv et al. 2007) (see Chapter 8). Because protein turnover (i.e., synthesis and breakdown) and amino acid and nitrogen metabolism are energy dependent, energy balance will have a significant impact on protein utilization and thus protein recommendations for female athletes. Negative energy balance (i.e., energy intake less than energy expenditure) causes a decrease in nitrogen balance and an increase in dietary protein requirements. Conversely, nitrogen balance improves with increasing energy intake for any given amount of protein consumed. It should be noted that increasing protein intake while at a constant energy intake does not improve nitrogen balance or protein utilization. As a result, energy balance, or the consumption of adequate calories to meet those expended, is likely more important to protein metabolism, especially when protein intakes are at the lower range of the DRIs (National Research Council 2005). Because exercise training contributes to energy expenditure, every effort must be made to replace the calories expended in exercise and maintain energy balance in order to optimize protein utilization (see Chapter 9). Female athletes, as well as their coaches, need to appreciate that energy is necessary to support protein synthesis for anabolism to occur such that gains in lean body mass are achieved. Conversely, restricting calorie intake to accomplish reductions in body mass should be reasonable and combined with sufficient protein intake such that fat is lost and muscle mass is maintained.

SUMMARY AND RECOMMENDATIONS

Protein is essential to the diets of healthy, physically active women. An adequate intake of protein in general, and the essential amino acids specifically, is important to maximize rates of protein synthesis, minimize protein catabolism, and optimize

protein utilization in response to habitual participation in both resistance and endurance exercise. Female athletes should strive to consistently consume protein within the recommended range of 1.2 to 1.7 $g \cdot kg^{-1} \cdot day^{-1}$. In doing so, attention should be given to distributing protein consumption throughout the day's meals and to incorporating protein into nutritional snacks or beverages consumed within the proximity of a strength-training session or endurance exercise bout of prolonged duration (Paddon-Jones and Rasmussen 2009). When appropriate, protein may be an additional component of a nutrition plan developed for nutrition support of ultra-endurance athletes during events. The role of energy balance in protein metabolism should also not be overlooked. Protein synthesis requires energy, and negative energy balance has been shown to result in a net negative protein balance. Therefore, attention should be given to consumption of sufficient calories for either weight maintenance or weight gain if amino acids are to be directed to synthetic (i.e., anabolic) rather than catabolic processes. When negative energy balance is intended for the purpose of weight loss, additional protein in the diet is likely beneficial to minimize losses in lean body mass. Female athletes who consume adequate protein *and* energy will enhance both health and performance outcomes subsequent to maintaining the quantity and quality of their lean body mass.

REFERENCES

Anthony, J. C., T. G. Anthony, et al. 2000. Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr* 130(2): 139–145.

Anthony, J. C., T. G. Anthony, et al. 2001. Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *J Nutr* 131(3): 856S–860S.

Bell, J. A., S. Fujita, et al. 2005. Short-term insulin and nutritional energy provision do not stimulate muscle protein synthesis if blood amino acid availability decreases. *Am J Physiol Endocrinol Metab* 289(6): E999–E1006.

Biolo, G., K. D. Tipton, et al. 1997. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 273(1 Pt 1): E122–E129.

Bolster, D. R., M. A. Pikosky, et al. 2005. Dietary protein intake impacts human skeletal muscle protein fractional synthetic rates after endurance exercise. *Am J Physiol Endocrinol Metab* 289(4): E678–E683.

Borsheim, E., Aarsland, A., and R. R. Wolfe. 2004. Effect of an amino acid, protein, and carbohydrate mixture on net muscle protein balance after resistance exercise. *Int J Sport Nutr Exerc Metab* 14(3): 255–271.

Breen, L., and S. M. Phillips. 2012. Nutrient interaction for optimal protein anabolism in resistance exercise. *Curr Opin Clin Nutr Metab Care* [Epub ahead of print February 23].

Burd, N. A., J. E. Tang, et al. 2009. Exercise training and protein metabolism: Influences of contraction, protein intake, and sex-based differences. *J Appl Physiol* 106(5): 1692–1701.

Bush, J. A., S. R. Kimball, et al. 2003. Translational control of protein synthesis in muscle and liver of growth hormone-treated pigs. *Endocrinology* 144(4): 1273–1283.

Carbone, J. W., J. P. McClung, et al. 2012. Skeletal muscle responses to negative energy balance: Effects of dietary protein. *Adv Nutr* 3(2): 119–126.

Carraro, F., W. H. Hartl, et al. 1990. Whole body and plasma protein synthesis in exercise and recovery in human subjects. *Am J Physiol* 258(5 Pt 1): E821–E831.

Cermak, N. M., A. S. Solheim, et al. 2009. Muscle metabolism during exercise with carbohydrate or protein-carbohydrate ingestion. *Med Sci Sports Exerc* 41(12): 2158–2164.

Du, J., and W. E. Mitch. 2005. Identification of pathways controlling muscle protein metabolism in uremia and other catabolic conditions. *Curr Opin Nephrol Hypertens* 14(4): 378–382.

Du, J., X. Wang, et al. 2004. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. *J Clin Invest* 113(1): 115–123.

Ferrando, A. A., K. D. Tipton, D. Doyle, S. M. Phillips, J. Cortiella, and R. R. Wolfe. 1998. Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. *Am J Physiol* 275:E864–E871.

Fujita, S., H. C. Dreyer, et al. 2007. Nutrient signalling in the regulation of human muscle protein synthesis. *J Physiol* 582(Pt 2): 813–823.

Gaine, P. C. 2005. Effects of endurance exercise on skeletal muscle protein turnover and intracellular signaling in female runners. Nutritional Sciences, Storrs, University of Connecticut. PhD dissertation: 285.

Gaine, P. C., M. Pikosky, D. Bolster, W. F. Martin, C. Maresh, and N. R. Rodriguez. 2007. Whole body protein turnover post-exercise in response to three levels of protein intake. *Med Sci Sports Exer* 39(3): 480–486.

Gaine, P. C., M. A. Pikosky, W. F. Martin, D. R. Bolster, C. M. Maresh, and N. R. Rodriguez. 2006. Level of dietary protein impacts whole body protein turnover in trained males at rest. *Metabolism* 55: 501–507.

Gibala, M. J. 2007. Protein metabolism and endurance exercise. *Sports Med* 37(4–5): 337–340.

Gontzea, I., P. Sutzescu, and S. Dumitrach. 1975. The influence of adaptation to physical effort on nitrogen balance in man. *Nutr Report Inter* 11: 231–236.

Hartman, J. W., D. R. Moore, et al. 2006. Resistance training reduces whole-body protein turnover and improves net protein retention in untrained young males. *Appl Physiol Nutr Metab* 31(5): 557–564.

Haub, M. D., A. M. Wells, M. A. Tarnopolsky, et al. 2002. Effect of protein source on resistive-training—Induced changes in body composition and muscle size in older men. *Am J Clin Nutr* 76(3): 511–517.

Hausswirth, C., and Y. Le Meur. 2011. Physiological and nutritional aspects of post-exercise recovery: Specific recommendations for female athletes. *Sports Med* 41(10): 861–882.

Hinton, P. S., T. C. Sanford, M. M. Davidson, O. F. Yakushko, and N. C. Beck. 2004. Nutrient intakes and dietary behaviors of male and female collegiate athletes. *Int J Sport Nutr Exerc Metab* 14(4): 389–405.

Howarth, K. R., S. M. Phillips, et al. 2010. Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. *J Appl Physiol* 109(2): 431–438.

Kerksick, C., T. Harvey, et al. 2008. International Society of Sports Nutrition position stand: Nutrient timing. *Int Soc Sports Nutr* 5: 17–29.

Kim, P. L., R. S. Staron, et al. 2005. Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *J Physiol* 568(Pt 1): 283–290.

Kimball, S. R. 2007. The role of nutrition in stimulating muscle protein accretion at the molecular level. *Biochem Soc Trans* 35(Pt 5): 1298–1301.

Kimball, S. R., P. A. Farrell, et al. 2002. Invited review: Role of insulin in translational control of protein synthesis in skeletal muscle by amino acids or exercise. *J Appl Physiol* 93(3): 1168–1180.

Koopman, R., A. Wagenmakers, et al. 2005. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis *in vivo* in male subjects. *Am J Phys Endocrinol Metab* 288: E645–E653.

Laidlaw, S. A., and J. D. Kopple. 1987. Newer concepts of the indispensable amino acids. *Am J Clin Nutr* 46: 593–605.

Lamont, L. S., A. J. McCullough, et al. 2001. Gender differences in leucine, but not lysine, kinetics. *J Appl Physiol* 91(1): 357–362.

Lamont, L. S., A. J. McCullough, et al. 2003. Gender differences in the regulation of amino acid metabolism. *J Appl Physiol* 95(3): 1259–1265.

Larson-Meyer, D. Enette. 2007. *Vegetarian Sports Nutrition*. Champaign, IL: Human Kinetics.
Lecker, S. H., A. L. Goldberg, et al. 2006. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. *J Am Soc Nephrol* 17(7): 1807–1819.
Lee, S. W., G. Dai, et al. 2004. Regulation of muscle protein degradation: Coordinated control of apoptotic and ubiquitin-proteasome systems by phosphatidylinositol 3 kinase. *J Am Soc Nephrol* 15(6): 1537–1545.
Lemon, P. W. 1998. Effects of exercise on dietary protein requirements. *Int J Sport Nutr* 8(4): 426–447.
Lemon, P. W. 2000. Beyond the zone: Protein needs of active individuals. *J Am Coll Nutr* 19(5 Suppl): 513S–521S.
Lemon, P. W., and F. J. Nagle. 1981. Effects of exercise on protein and amino acid metabolism. *Med Sci Sports Exerc* 13(3): 141–149.
Levenhagen, D. K., C. Carlson, et al. 2002. Postexercise protein intake enhances whole-body and leg protein accretion in humans. *Med Sci Sports Exerc* 34(5): 828–837.
Lunn, W. R., S. M. Pasiakos, et al. 2012. Chocolate milk and endurance exercise recovery: Protein balance, glycogen, and performance. *Med Sci Sports Exerc* 44(4): 682–691.
McKenzie, S., S. M. Phillips, et al. 2000. Endurance exercise training attenuates leucine oxidation and BCOAD activation during exercise in humans. *Am J Physiol Endocrinol Metab* 278(4): E580–E587.
Miller, S., P. C. Gaine, et al. 2007. Nutritional supplementation throughout an endurance run effects leucine kinetics during recovery. *Int J Sport Nutr Exerc Metab* 17(5): 456–467.
Moore, D. R., M. J. Robinson, et al. 2009. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 89(1): 161–168.
National Research Council. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients).* Washington, DC: The National Academies Press.
Nattiv, A., A. B. Loucks, M. M. Manore, C. F. Sanborn, J. Sundgot-Borgen, and M. P. Warren. 2007. American College of Sports Medicine position stand: The female athlete triad. *Med Sci Sports Exerc* 39(10): 1867–1882.
Paddon-Jones, D., and B. B. Rasmussen. 2009. Dietary protein recommendations and the prevention of sarcopenia. *Curr Opin Clin Nutr Metab Care* 12(1): 86–90.
Pasiakos, S. M., L. M. Vislocky, et al. 2010. Acute energy deprivation affects skeletal muscle protein synthesis and associated intracellular signaling proteins in physically active adults. *J Nutr* 140(4): 745–751.
Phillips, S. M. 2004. Protein requirements and supplementation in strength sports. *Nutrition* 20(7–8): 689–695.
Phillips, S. M. 2008. Insulin and muscle protein turnover in humans: Stimulatory, permissive, inhibitory, or all of the above? *Am J Physiol Endocrinol Metab* 295(4): E731.
Phillips, S. M. 2010. The science of muscle hypertrophy: Making dietary protein count. *Proc Nutr Soc* 70(1): 100–103.
Phillips, S. M., S. A. Atkinson, et al. 1993. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J Appl Physiol* 75(5): 2134–2141.
Phillips, S. M., G. Parise, et al. 2002. Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state. *Can J Physiol Pharmacol* 80(11): 1045–1053.
Phillips, S. M., K. D. Tipton, et al. 1997. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol* 273(1 Pt 1): E99–E107.
Phillips, S. M., K. D. Tipton, et al. 1999. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am J Physiol* 276(1 Pt 1): E118–E124.
Phillips, S. M., and L. J. Van Loon. 2011. Dietary protein for athletes: From requirements to optimum adaptation. *J Sports Sci* 29(Suppl 1): S29–S38.

Pikosky, M. A., P. C. Gaine, et al. 2006. Aerobic exercise training increases skeletal muscle protein turnover in healthy adults at rest. *J Nutr* 136(2): 379–383.

Reeds, P. J., and P. J. Garlick. 1984. Nutrition and protein turnover in man. *Adv Nutr Res* 6: 93–138.

Rodriguez, N. R. 2009. Making room for protein in approaches to muscle recovery from endurance exercise. *J Appl Physiol* 106(4): 1036–1037.

Rodriguez, N. R., N. M. DiMarco, et al. 2009. 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): 509–527.

Rodriguez, N. R., L. M. Vislocky, et al. 2007. Dietary protein, endurance exercise, and human skeletal-muscle protein turnover. *Curr Opin Clin Nutr Metab Care* 10(1): 40–45.

Rowlands, D. S., and D. P. Wadsworth. 2011. Effect of high-protein feeding on performance and nitrogen balance in female cyclists. *Med Sci Sports Exerc* 43(1): 44–53.

Saunders, M. J., N. D. Luden, et al. 2007. Consumption of an oral carbohydrate-protein gel improves cycling endurance and prevents postexercise muscle damage. *J Strength Cond Res* 21(3): 678–684.

Tarnopolsky, M. 2004. Protein requirements for endurance athletes. *Nutrition* 20(7–8): 662–668.

Tarnopolsky, L. J., J. D. MacDougall, S. A. Atkinson, M. A. Tarnopolsky, and J. R. Sutton. 1990. Gender differences in substrate for endurance exercise. *J Appl Physiol* 68(1): 302–308.

Tipton, K. D., T. A. Elliott, et al. 2007. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *Am J Phys Endocrinol Metab* 292(1): E71–E76.

Tipton, K. D., and A. A. Ferrando. 2008. Improving muscle mass: Response of muscle metabolism to exercise, nutrition and anabolic agents. *Essays Biochem* 44: 85–98.

Tipton, K. D., B. E. Gurkin, et al. 1999. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J Nutr Biochem* 10(2): 89–95.

Tipton, K. D., B. B. Rasmussen, et al. 2001. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab* 281(2): E197–E206.

Tipton, K. D., and O. C. Witard. 2007. Protein requirements and recommendations for athletes: Relevance of ivory tower arguments for practical recommendations. *Clin Sports Med* 26(1): 17–36.

Tipton, K. D., and R. R. Wolfe. 2001. Exercise, protein metabolism, and muscle growth. *Int J Sport Nutr Exerc Metab* 11(1): 109–132.

Todd, K. S., G. E. Butterfield, et al. 1984. Nitrogen balance in men with adequate and deficient energy intake at three levels of work. *J Nutr* 114(11): 2107–2118.

Toth, M. J., C. K. Sites, D. E. Matthews, and P. R. Casson. 2006a. Ovarian suppression with gonadotropin-releasing hormone agonist reduces whole body protein turnover in women. *Am J Physiol Endocrinol Metab* 291(3):E483–E490.

Toth, M. J., C. K. Sites, and D. E. Matthews. 2006b. Role of ovarian hormones in the regulation of protein metabolism in women: Effects of menopausal status and hormone replacement therapy. *Am J Physiol Endocrinol Metab* 291(3):E639–E646.

Valentine, R. J., M. J. Saunders, et al. 2008. Influence of carbohydrate-protein beverage on cycling endurance and indices of muscle disruption. *Int J Sport Nutr Exerc Metab* 18(4): 364–378.

Van Essen, M., and M. J. Gibala. 2006. Failure of protein to improve time trial performance when added to a sports drink. *Med Sci Sports Exerc* 38(8): 1476–1483.

Venderley, A. M., and W. W. Campbell. 2006. Vegetarian diets: Nutritional considerations for athletes. *Sports Med* 36(4):293–305.

Vislocky, L. M., P. C. Gaine, et al. 2008. Gender impacts the post-exercise substrate and endocrine response in trained runners. *J Int Soc Sports Nutr* 5: 7.

Wolfe, Robert R. 2006. The underappreciated role of muscle in health and disease. *Am J Clin Nutr* 84(3): 475–482.

Wolfe, Robert R., and David L. Chinkes. 2004. *Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis,* 2nd edition. Hoboken, NJ: John Wiley & Sons.

4 Fluid and Electrolyte Requirements for Female Athletes

Megan M. Wenner and Nina S. Stachenfeld

CONTENTS

INTRODUCTION

Muscle activity during exercise generates significant heat production creating a challenge for the body's temperature regulating system even under mild conditions. Because sweat evaporation is the primary method for humans to cool the body, athletes can lose significant amounts of water during exercise. Fluid losses vary greatly across different sports and among individual athletes, ranging from 0.5 to 2.6 L/h. Sweat losses are even greater when exercise is performed in hot and humid conditions. Depending on the magnitude of the sweating response and drinking behavior of the athlete, fluid imbalances such as dehydration and hyponatremia can develop, leading to poor performance, increased risks of heat exhaustion, heatstroke, and in extreme circumstances, brain damage or even death.

Fluid homeostasis encompasses balance (in and out) and regulation (how the body responds to changes in different exposures, such as changes in the external environment to the hormonal environment). Regulation can shift operating points around which fluid or tonicity (in this case sodium concentration) is regulated without altering balance or a need for altering requirements. Research suggests that

there are sex differences when it comes to fluid regulation, which could potentially affect fluid balance and thus might have a bearing on fluid requirements for female athletes. This chapter will review the basic concepts of fluid balance and fluid regulation, examine sex differences in thermoregulation and fluid regulation, and discuss whether these differences necessitate distinct fluid recommendations for female athletes.

OVERVIEW OF FLUID BALANCE AND REGULATION

Approximately 60 to 65% of the human body is composed of water. Body fluids are stored in two compartments: inside the cells (intracellular ~60%) and outside the cells (extracellular ~40%). The extracellular compartment is further subdivided into the intravascular and interstitial fluid compartments, which make up ~20 and 80%, respectively. Fluid within cells and outside of the vascular compartment cannot be immediately accessed during exercise, thus only the plasma volume is available for sweating and thermoregulation during exercise.

Changes in body water and solute concentrations can exert profound effects on cellular and organ system function. Thus, sophisticated regulatory mechanisms have evolved to maintain body fluid volume and composition despite sudden fluxes in water intake or loss. The regulatory mechanisms involve reflexes whose receptors within the vasculature, brain, and gut are sensitive to mechanical and chemical changes that occur during shifts in water and electrolyte content, and whose effector systems act to modify rates of fluid intake and fluid output. For example, dehydration (hyperosmotic hypovolemia) leads to the sensory and behavioral responses of thirst and fluid intake and the physiological responses of sodium and water retention by the kidney (Figure 4.1). On the other end of the spectrum, under certain circumstances, a small percentage of athletes (1 to 13%) can retain water during exercise leading to a fall in plasma sodium concentration. This condition occurs when athletes competing in long-duration events ingest hypotonic fluids (fluids with lower sodium concentration than is in the blood) in greater excess than they are able to excrete (hypervolemic hyponatremia).

EFFECTS OF EXERCISE ON FLUID BALANCE AND REGULATION

Fluid and electrolyte requirements during exercise are very much dependent on the interaction between the ambient temperature and the type and intensity of the exercise. In turn, body temperature regulation interacts with systems that regulate volume and osmotic pressure of the extracellular fluid (Morimoto 1990). For example, the severity of dehydration can be affected by initial blood volume and plasma sodium concentration (Nose et al. 1988, 1990). Specifically, blood volume expansion, which increases the amount of water available for sweating, improves the cardiovascular and thermoregulatory responses during physical activity (Fortney et al. 1983). The likely reason for the dependence of heat transfer on absolute blood volume during exercise in the heat is that the ability of the heart to pump blood to the skin, and therefore provide increased convective heat transfer from the body core to the skin, is a function of preload. When blood volume is expanded, cardiac stroke volume

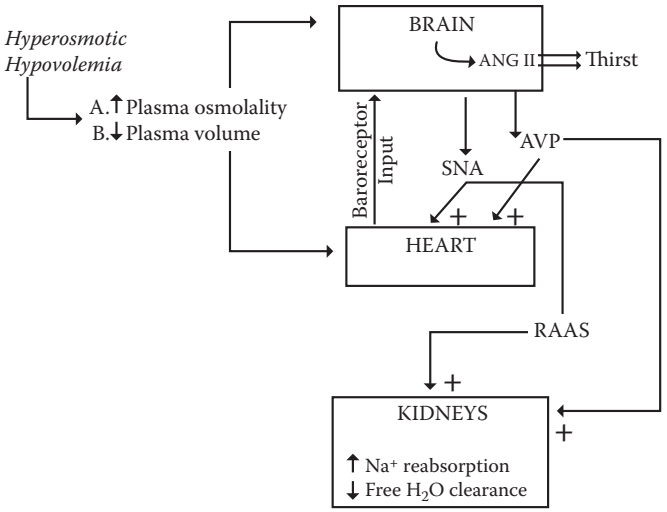


FIGURE 4.1 Central and peripheral control of thirst and fluid balance. Exercise-induced dehydration (hyperosmotic hypovolemia) causes an increase in plasma osmolality and a decrease in plasma volume. Hyperosmolality is sensed by osmoreceptors in the circumventricular organs of the brain; hypovolemia is sensed by low-pressure baroreceptors of the heart, which send signals via nerves to the brain. (For detailed information on neural circuitry see Stachenfeld, N. S., Acute Effects of Sodium Ingestion on Thirst and Cardiovascular Function, *Curr Sports Med Rep* 7: S7–S13, 2008; Toney, G. M., and Stocker, S. D., Hyperosmotic Activation of CNS Sympathetic Drive: Implications for Cardiovascular Disease, *J Physiol* 588: 3375–3384, 2011.) In response to both signals, there is an increase in thirst sensation and drinking. Also in response to osmo- and baroreceptor activation, there is an increase in sympathetic nervous system activation (SNA) and an increase in the release of arginine vasopressin (AVP) leading to elevations in heart rate, peripheral vasoconstriction, plasma renin activity, and renin-angiotensin-aldosterone system (RAAS) activation. Activation of the RAAS and AVP increase renal sodium and free water reabsorption and thus overall body fluid retention. Also sensitive to changes in body water and tonicity is angiotensin II (ANG II) in the brain, triggering thirst. When water and sodium are available, consumption will return the body to euhydration.

increases, resulting in elevated cardiac output and improved ability to deliver blood to muscle and skin simultaneously, where heat transfer takes place. Conversely, blood volume contraction results in a gradual fall in preload during exercise (Tripathi et al. 1990), a reduction in cardiac output, and an associated increase in skin vascular resistance at any internal temperature, explaining the decrease in heat transfer.

The temperature threshold for the onset of a thermoregulatory effector response (i.e., sweating and peripheral vasodilation) is defined as the core temperature above which the effector response is greater than that of baseline. A shift in the core temperature threshold is often referred to as a change in the set point for temperature regulation. A reduction in the set point for temperature regulation secondary to blood volume expansion improves exercise performance in the heat because more water is

available for sweat evaporation. Because the body temperature is maintained at a lower level, strain on the cardiovascular system is reduced; for example, under these conditions, heart rate is lower. Conversely, dehydration and the associated plasma volume loss elevate exercise core temperature (Sawka and Pandolf 1990; Sawka et al. 1992), increases cardiovascular strain, and decreases exercise tolerance (Sawka and Wenger 1988; Sawka et al. 1992).

SEX DIFFERENCES IN TEMPERATURE AND FLUID REGULATION

Most sex differences in thermoregulation can be explained by differences in body size, body composition, and fitness level between men and women (Kenny et al. 2008; Gagnon et al. 2008, 2009). Because women are generally smaller and have less lean body mass (both in absolute and relative amounts) than men, their fluid and electrolyte losses are typically lower. The differences in sweat and electrolyte losses can also impact thermoregulation, but the magnitude of the sex differences is very much dependent on the external environment. Because the primary mechanism for heat loss in humans is evaporation, the heat loss system during exercise is limited by the capacity of the circulation to deliver heat to the skin for dissipation and the sweat glands to secrete water for evaporation. Due to their smaller body size and surface area available for sweating, women typically have lower sweating rates and electrolyte losses compared to men (Shapiro et al. 1980; Sawka et al. 2007). The more important sex differences in thermoregulation appear during exercise in a hot wet environment, where women have lower sweating rates at similar core temperatures because of their greater body surface area to body weight ratio compared to that of men (Shapiro et al. 1980). In other words, compared to their body weight, women have a greater skin area that is available for sweating relative to their body weight, which is an advantage in humid environments when evaporative sweating can be suppressed. Thus, women have a distinct fluid regulatory advantage in the hot, wet environment because they maintain core temperature while losing less fluid through sweat. This advantage is related to the fact that heat production is primarily weight dependent, whereas evaporation or cooling depends primarily on body surface area (BSA) (i.e., skin surface). In hot, wet environments evaporative sweating is limited so the greater surface area for sweating in men confers little thermoregulatory advantage. In women, their lower exercising lean mass produces less heat, which attenuates the rise in core temperature. Conversely, in a hot, dry environment, a larger BSA available for sweating should confer an advantage for evaporative sweating and heat loss through convection. Under these circumstances, men have greater heat production because of their size but also have greater surface area with which to evaporate sweat. Women produce less heat compared to men because of their smaller lean mass, and also lose less fluid and electrolytes through sweating. Under most circumstances core temperature is maintained similarly in both sexes, but slower sweating rates in women result in lower fluid and electrolyte requirements. Under both hot, wet and hot, dry conditions, this lower sweat loss reduces the need to ingest fluids and may contribute to the greater risk of hyponatremia in women.

During cycling exercise, men have higher sweat rates compared to women, despite similar *ad libitum* fluid intake suggesting more efficient sweat production and evaporation and a greater reserve capacity for increased sweating (Carter et al. 2005). In earlier studies, differences in fitness between men and women may have played a role in differences in sweating efficiency. However, even when fitness is similar between the sexes, men still have slightly higher sweating rates than women (Millard-Stafford et al. 1995). Hydration status and exercise performance in similarly trained men and women in a hot, humid environment were compared during a simulated 40K race in the humid heat. Although the running times, fluid intake, and sweating rates were similar between the men and women, the women had lower plasma volume losses and higher serum potassium and sodium concentrations than the men during exercise, and core temperature was also lower in the women.

The study of sex differences in fluid regulation is complex in young men and women because reproductive hormones have profound effects on fluid regulation and fluctuate monthly in women over the course of the menstrual cycle (Figure 4.2). Estrogen with and without progesterone exposure alters the threshold for thermoregulation during exercise in the heat. These changes in thermoregulatory response may be a function of the plasma volume expansion associated with high levels of estrogen in the blood and tissue (Tankersley et al. 1992; Stachenfeld, Silva et al. 1999). Core temperature responses to passive heating and exercise in heat are reduced during the midfollicular phase of the menstrual cycle, the cycle phase characterized by rising estrogen levels (Horvath and Drinkwater 1982; Hirata et al. 1986; Kolka and Stephenson 1989; Pivarnik et al. 1992). An early study by Haslag and Hertzman (1965) demonstrated that the onset of thermoregulatory sweating during whole-body heating occurred at a lower core temperature in women during their follicular phase relative to the luteal phase. Stephenson and Kolka followed up these findings demonstrating earlier (lower) core temperature thresholds for both sweating and cutaneous vasodilation in a hot environment during exercise in the midfollicular phase compared to the midluteal phase (Stephenson and Kolka 1999). The relationship between reproductive hormone exposure and thermoregulation appears to continue through the postmenopausal years. Tankersley et al. (1992) demonstrated that the thresholds for the onset of sweating and vasodilation were reduced by 0.47°C and 0.48°C respectively, following 2 weeks of estrogen replacement therapy in postmenopausal women. Taken together, these data show strong evidence that estrogen exposure is associated with a lower thermoregulatory operating point during exercise and suggests that these changes may be related to plasma volume expansion (Stachenfeld, Silva et al. 1999).

During the luteal phase, when both estrogen and progesterone exposures are high, the core temperature threshold is shifted to the right during exercise in the heat (Figure 4.3) (Stachenfeld et al. 2000). Using oral contraceptives, we found that unopposed progestin administration caused plasma volume contraction concomitant with increased regulated body temperature as reflected by increases in both basal core temperature and delayed (higher) core temperature threshold for sweating (Figure 4.3). Moreover, estradiol administered with progestin restored plasma volume and reversed these thermoregulatory changes (Figure 4.3) (Stachenfeld et al. 2000).

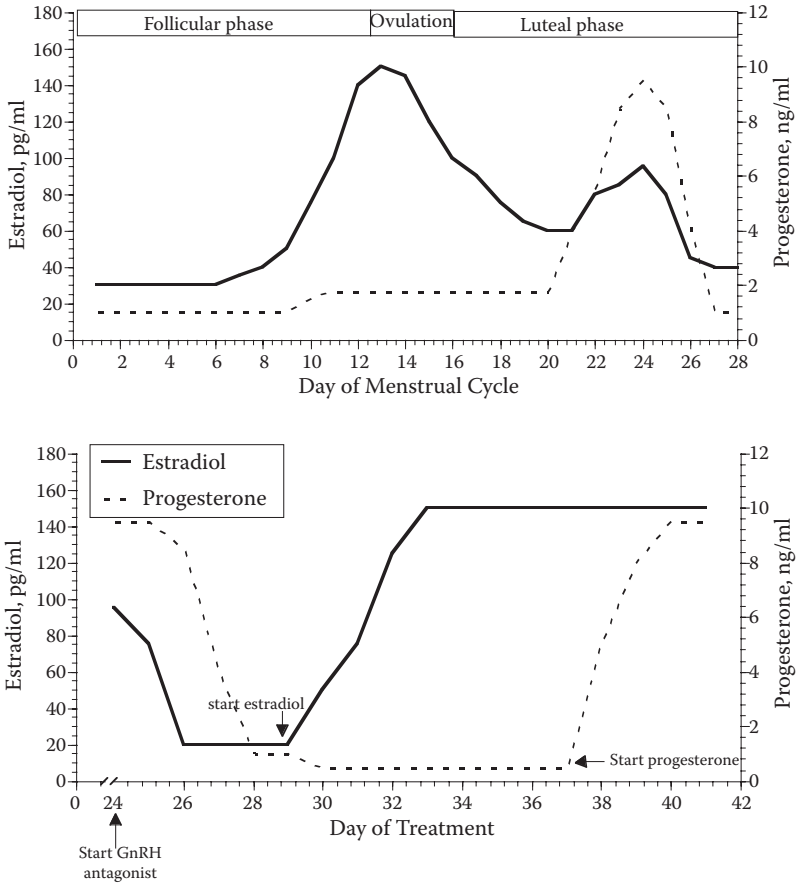


FIGURE 4.2 Changes in 17β-estradiol and progesterone across the menstrual cycle (top). Changes in 17β-estradiol and progesterone during treatment with a gonadotropin-releasing hormone (GnRH) antagonist beginning on day 25 of a normal menstrual cycle, followed by treatment with two 17β-estradiol patches (0.1 mg) and oral progesterone (200 mg/day) (bottom). (From Stachenfeld, N. S., and Taylor, H. S., Sex Hormone Effects on Body Fluid and Sodium Regulation in Women with and without Exercise-Associated Hyponatremia, *J Appl Physiol* 107: 864–872, 2009.)

In sum, the hormone-related shifts in core temperature thresholds for sweating indicate thermoregulatory sweating onset at a lower core temperature during estrogen exposure. This shift improves thermoregulation, but potentially at the expense of greater body water loss. In contrast, the contracted plasma volume associated with progesterone exposure impairs thermoregulatory function. However, as will be shown in the following sections, these changes in sweating do not consistently alter performance, fluid balance, or health risks in women (Stephenson and Kolka 1985; Kolka and Stephenson 1989; Stachenfeld et al. 2000).

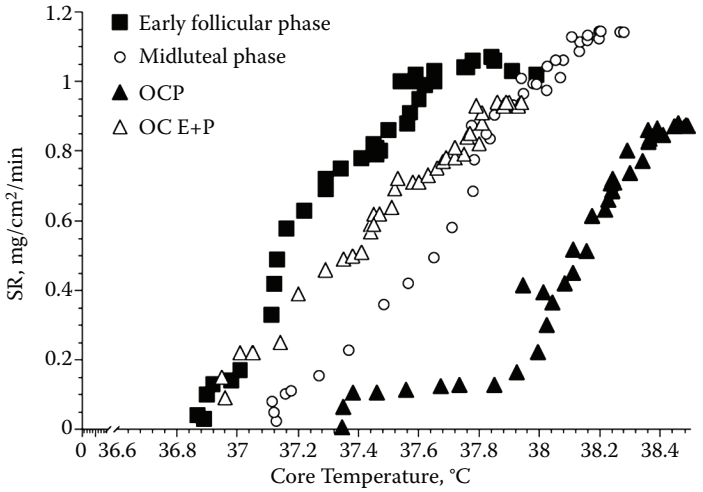


FIGURE 4.3 Sweating rate (SR) as a function of core (esophageal) temperature during 40 minutes of exercise in the heat in young, healthy women during the early follicular, midluteal phase, progestin-only (OCP) and combined progestin + ethinyl estradiol (OC E+P) oral contraceptives. (Data are based on Stachenfeld, N. S., Silva, C. S., and Keefe, D. L., Estrogen Modifies the Temperature Effects of Progesterone, *J Appl Physiol* 88: 1643–1649, 2000.)

Sex Hormones and Fluid Regulation

Dehydrating exercise causes plasma volume loss concomitant with increases in plasma tonicity, stimulating both the volume and osmotic receptors. Thus, research has examined the effects of the sex hormones estradiol and progesterone on the osmotic regulation of thirst and arginine vasopressin, which is the primary hormone involved in the regulation of renal free water. Stachenfeld, Silva et al. (1999) found that estrogen and progesterone exposures were associated with earlier osmotic thresholds for AVP during dehydrating exercise (Figure 4.4). That is, a lower plasma osmolality was required to induce AVP release, which should lead to an increase in water retention by the kidney. Interestingly, although thirst sensation was more sensitive to the osmotic stimulation induced by exercise dehydration during high estrogen exposures (Figure 4.5), the subjects drank the same amount of water when given the opportunity to drink freely (Stachenfeld, Silva et al. 1999). Finally, fluctuations of estrogens and progesterone across the menstrual cycle also influence sodium-regulating hormone responses during exercise (De Souza et al. 1989). Typically, increases in fluid-regulating hormones like AVP and sodium-regulating hormones like renin and aldosterone lead to water and fluid retention, respectively, by the kidney. Nonetheless, despite these changes in the fluid- and sodium-regulating hormones and thirst sensation, there does not seem to be meaningful effects of sex hormone exposure on whole-body fluid and sodium regulation with exercise (Stachenfeld, Silva et al. 1999).

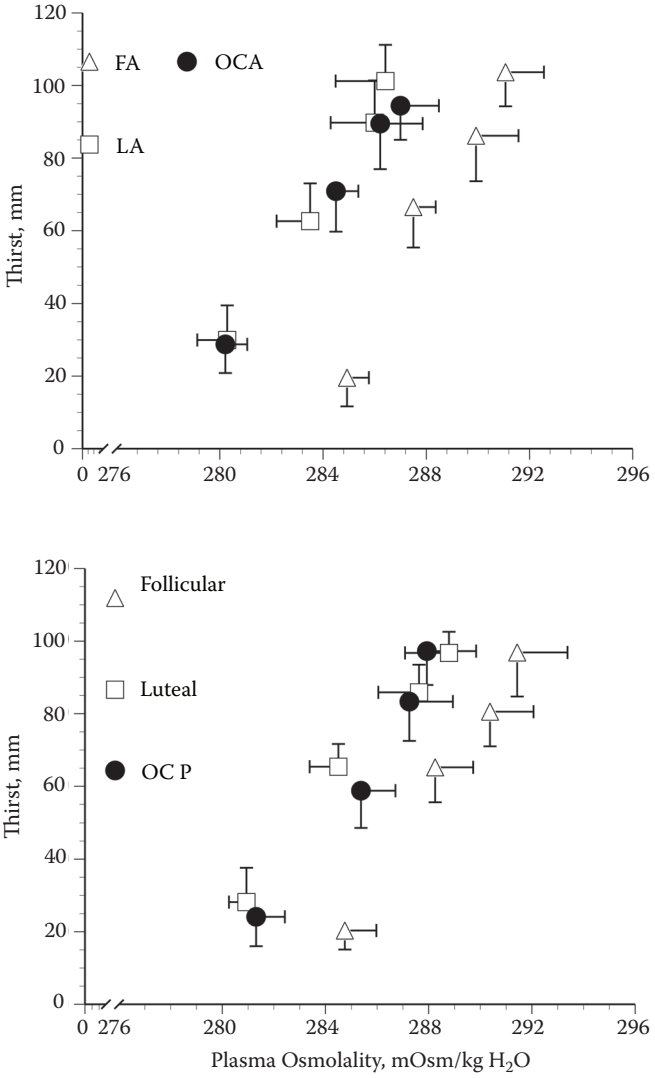


FIGURE 4.4 Plasma arginine vasopressin (AVP) concentration as a function of plasma osmolality during 120 min of exercise-induced dehydration in the follicular and luteal phases of the menstrual cycle and during combined (ethinyl estradiol + progestin, OC E+P) and progestin-only (OC E+P) oral contraceptive administration. Note the high progesterone/progestin conditions (luteal, OC E+P, OC P) shifted the $P_{[AVP]}$-P_{Osm} curves to the left relative to the follicular phase. (Reprinted from Stachenfeld, N. S. et al., Effects of Oral Contraceptives on Body Fluid Regulation, *J Appl Physiol* 87: 1016–1025, 1999. Copyright © 1999 The American Physiological Society. Used with permission.)

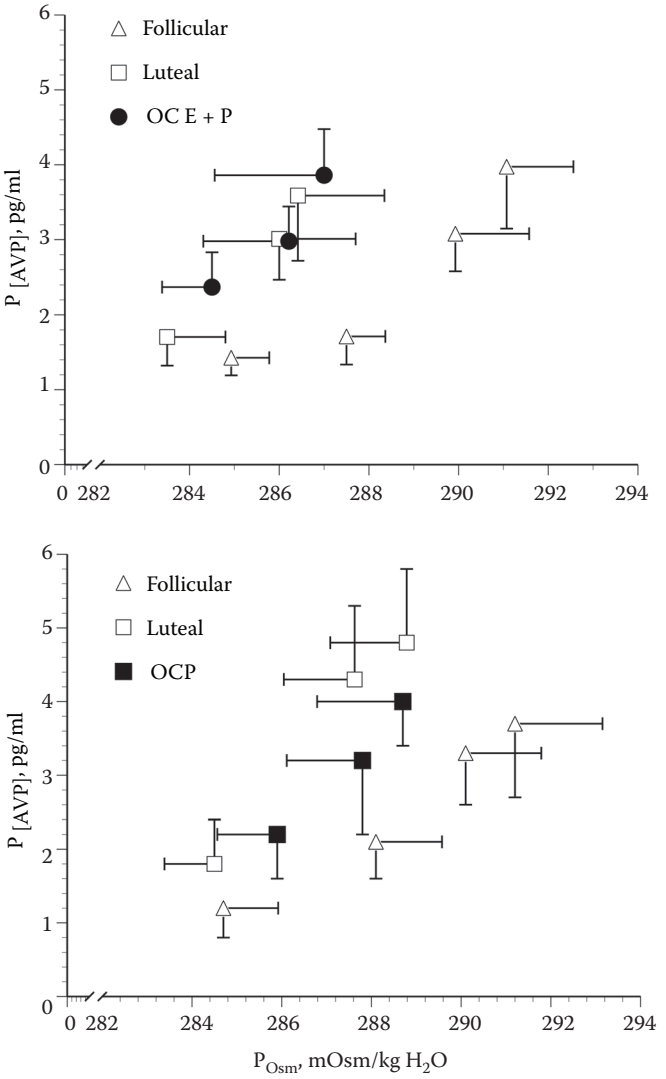


FIGURE 4.5 Thirst as a function of plasma osmolality during 120 min of exercise-induced dehydration in the follicular and luteal phases of the menstrual cycle and during combined (ethinyl estradiol + progestin, OC E+P) and progestin-only (OC P) oral contraceptive administration. Note the high progesterone/progestin conditions (luteal, OC E+P, OC P) shifted the thirst-P_{Osm} curves to the left relative to the follicular phase. (Based on data from Stachenfeld, N. S. et al., Effects of Oral Contraceptives on Body Fluid Regulation. *J Appl Physiol* 87: 1016–1025, 1999.)

In addition to changes in endogenous reproductive hormone exposure and their effects on fluid and electrolyte balance over the course of the menstrual cycle, administration of exogenous estrogens and progesterone (i.e., via the use of oral contraceptives) can cause additional alterations. For example, sex hormone administration is accompanied by significant sodium retention (Aitken et al. 1974; Blahd et al. 1974), resulting in the plasma volume expansion described earlier (Whitten and Bradbury 1951; Barron et al. 1986; Tankersley et al. 1992; Baker et al. 1994). Oral contraceptive agents, which deliver pharmacological levels of estrogens, increase total body water (Blahd et al. 1974). In fact, variations in plasma volume at rest and during exercise, which are observed following estrogen administration and during different phases of the menstrual cycle are comparable in magnitude to the reported effects of posture, skin temperature, and exercise intensity (Harrison 1985).

The studies described above were limited in their ability to isolate the individual effects of estrogens or progesterone because the natural levels of these hormones fluctuate throughout the menstrual cycle and oral contraceptives are usually provided with both estrogens and progestins. Therefore, in order to confirm the findings of the physiological effects of sex hormones on body fluid regulation, a series of studies that employed a different paradigm to control the levels of the female reproductive hormones was performed. These studies used either a gonadotropin-releasing hormone (the hormone responsible for triggering the menstrual cycle) agonist or antagonist. The agonist or antagonist suppresses reproductive function and thereby endogenous reproductive hormone production (Figure 4.2). The suppression was followed by administration of estradiol, progesterone, or combined estradiol-progesterone to physiological levels. This paradigm isolated the effects of these hormones on the fluid and sodium regulatory systems during acute fluid or osmotic challenges. Similar to studies examining body fluid regulation over the course of the menstrual cycle, the "suppression add-back" paradigm indicated that estradiol lowered and progesterone raised the threshold for osmotically induced AVP release and thirst onset, as well as altered the sodium regulation hormones (Stachenfeld and Keefe 2002). As with the studies described earlier, all of these physiological changes in regulation did not alter overall water and sodium regulation.

In sum, the collective data from research examining the effects of sex hormones on fluid and sodium regulation indicate that both the hormonal regulatory and sensory systems are affected by reproductive hormone status. Sex hormone changes across the menstrual cycle during oral contraceptive administration and during the suppression add-back paradigm have effects that are consistent with greater water and sodium retention. However, their associated physiological and behavioral systems are either unaffected or the changes are too small to have a significant impact on fluid regulation or performance. Thus, these reproductive hormones alter the homeostatic set point around which the system is regulated rather than induce excess fluid or sodium retention or loss.

Sex Differences in Sodium Balance and Regulation

Completion of a marathon, or similar endurance event, usually results in a body water loss of ~2 to 3%. Because the loss of body water generally exceeds the loss of sodium, plasma sodium concentrations are generally elevated at the end of endurance events

(by ~5 to 7 mmol/L). Nonetheless, under certain conditions, a small number of athletes may experience reductions in plasma sodium concentration during endurance exercise by as much as 5 mmol/L (Speedy et al. 2001; Almond et al. 2005) resulting in hyponatremia. Hyponatremia is generally defined as plasma sodium concentration of less than 135 mmol/L following an endurance event, although most athletes can tolerate such losses. This fall in plasma sodium concentration can be the result of ingestion of hypotonic fluids in excess of what the athlete is able to excrete (hypervolemic hyponatremia), or the result of unusually high sweat sodium concentration concomitant with large sweat volume losses (hypovolemic hyponatremia). Most athletes tolerate a substantial fall (>5 mmol/L) in plasma sodium concentration without symptoms (Speedy et al. 2001). However, in those athletes who cannot tolerate these large sodium and water losses, or when the hyponatremia is extreme (120 to 125 mmol/L) or prolonged, the consequences can be severe (cerebral edema and metabolic encephalopathy, permanent brain damage, death). Thus, although exercise-associated hyponatremia (EAH) is a much more rare occurrence than dehydration during endurance exercise, its consequences can be far more severe.

It appears as though women are at a greater risk for EAH compared to their male counterparts. The increased risk among women has been attributed to their lower body weight and size, lower sweating rates, excess water ingestion, and longer racing times relative to men (Speedy et al. 2001; Almond et al. 2005). A recent study in young women indicated that a previous hyponatremic episode is a risk factor for future EAH (Stachenfeld and Taylor 2009). Estradiol exposure may also be a risk factor for EAH in women because it is associated with greater free water retention and alterations in fluid distribution independent of body size or fluid intake behavior (Fraser and Arieff 1997; Ayus et al. 2000; Stachenfeld et al. 1996, 1998, 1999, 2001, 2003; Stachenfeld and Keefe 2002; Stachenfeld and Taylor 2004). Somewhat surprisingly, despite these previously defined relationships, sex hormone manipulation does not impact plasma sodium concentration in hyponatremic women during long-term exercise (Stachenfeld and Taylor 2009). It is, therefore, unlikely that taking estrogen, for example in oral contraceptives, increases EAH risks, although more research into this area is warranted.

Even though reproductive hormone exposure does not increase the incidence of EAH in women, estradiol may leave women more susceptible to the extreme consequences of hypervolemic hyponatremia. For example, a combination of anesthesia, postsurgical stress, and nausea can lead to dramatic increases in the water retention hormone, arginine vasopressin, in both men and women undergoing even minor surgery. However, the greater AVP exposure is associated with brain swelling and damage almost exclusively in women (Arieff 1986; Ayus et al. 1992; Ayus and Arieff 1996; Fraser and Arieff 1997). Studies in rats have demonstrated that in response to increasing hypotonic water retention, AVP increases brain capillary and cerebroventricular ependymal cell water permeability through specific water channels (aquaporin AQP4), which are regulated via AVP-V_1 receptors (Fraser et al. 1989). This alteration in cell water permeability leads to increases in sodium and water inside the brain cells and results in significant astrocyte swelling. In male animals, the Na^+-K^+ ATPase pump acts to extrude Na^+ out of brain cells to normalize volume (Fraser and Sarnacki 1989). However, this Na^+-K^+ ATPase pump action is

inhibited in females, especially during estradiol administration, which blocks astroglia regulatory volume decreases resulting in greater water remaining within the cells (Fraser and Sarnacki 1989). Thus, estradiol may play a significant role in the greater risk of cerebral edema and encephalopathy found in hyponatremic women, indicating a more complex etiology than simply lower body size, longer running times, and cultural norms of drinking behavior (Almond et al. 2005). These sex differences or estradiol effects have not been demonstrated during exercise or during recovery from exercise, but clearly would be difficult to study in humans.

Effects of Exercise Training on Fluid Requirements in Women

Chronic aerobic exercise results in a number of physiological alterations and adaptations that can alter fluid regulation. Exercise training and heat acclimation can increase sweating responses and reduce electrolyte sweat concentration. Specifically, exercise training increases the sensitivity (or slope) and reduces the thresholds of the relationships between core temperature, peripheral vasodilation, and sweating. These changes in the sweating response lead to greater fluid losses because the response is greater for a given increase in core temperature (sensitivity), and a smaller increase in internal temperature is required to induce sweating (threshold). These improvements occur similarly in men and women, reduce the sex differences in fluid and temperature regulation during exercise in the heat, and lower the hormone-induced shifts in thermoregulatory control described earlier (Kuwahara et al. 2005a, 2005b). Exercise training greatly improves thermoregulation because sweat evaporation is more efficient, but it increases the fluid requirements.

Limited available research indicates that men and women respond similarly in terms of thermoregulatory and fluid regulation responses to exercise training. For example, Roberts et al. (1977) demonstrated that 10 days of aerobic exercise training reduced the core temperature threshold for peripheral vasodilation and sweating similarly in men and women, permitting improved heat dissipation in both groups. Moreover, the improved thermoregulation was augmented if the exercise training was performed in the heat due to acclimatization. One key limitation among many of the studies examining sex differences in thermoregulatory and fluid regulation response to exercise is that they did not control for the phase of the menstrual cycle; thus, fluctuations in reproductive hormones that occur across the menstrual cycle were not considered.

While studying the effects of training on thermoregulation in women, Araki et al. (1981) measured sweating responses to exercise in a hot environment in trained and untrained women during the same phase of the menstrual cycle (within 7 days postovulation). The trained women demonstrated earlier onset of sweating compared to the untrained women during exercise. Following this baseline testing, the untrained women underwent 60 days of bicycle ergometer training, which improved thermoregulatory sweating responses. Interestingly, these menstrual cycle differences in sweating were not observed in trained women indicating that exercise training may attenuate menstrual phase differences in fluid and temperature regulation during exercise in the heat (Kuwahara et al. 2005a,b).

The greater sweating responses in athletically trained women result in greater body fluid losses. Thus, women athletes need to be cognizant that as they improve their fitness level, their fluid and electrolyte requirements may increase. Another important consideration is the lower sodium concentration of the sweat which can occur with training and heat acclimation, an adaptation that occurs to a similar extent in men and women. The lower concentration of sodium in sweat is an important training adaptation, because this lower electrolyte loss will balance the greater sweating rates achieved with training. Finally, even though sweat sodium concentration is generally reduced with training, these rates remain highly variable across individuals, varying as much as 10 to 70 mEq/L (Sawka et al. 2007).

Fluid and Electrolyte Recommendations for Female Athletes

The hormonal effects on thermoregulation as well as the regulation of fluid and sodium described above, while interesting, seem to have little practical impact on fluid and sodium balance during exercise and thus requirements for these nutrients for the woman athlete. Individual variability among women with regard to fitness level, body size, and temperature acclimatization as well as the external environment all play more significant roles in fluid and sodium requirements. As per the American College of Sports Medicine (ACSM) guidelines, fluid and sodium requirements must be based on individual assessments of the athlete's fluid and sodium losses with the goal of avoiding both dehydration and excess fluid ingestion (and perhaps EAH). Moreover, even though most general fluid and sodium replacement guidelines found within the ACSM position stand were based on data from men, women athletes can readily adapt these guidelines by correcting for their individual body weights (i.e., with the consideration that the average male body weight used in most calculations is ~70 kg.) The following paragraphs summarize the fluid and sodium recommendations for female athletes.

Pre-Exercise Hydration

While pre-exercise plasma or blood volume can impact performance, athletes do not gain an advantage by attempting to "overhydrate" and acutely expand central blood volume during the pre-exercise period. In fact, excessive fluid consumption (i.e., *hyperhydrating*) prior to exercise will result in increased urination and may increase the risk of hyponatremia. Thus, athletes should drink just enough to reach euhydration (thus adequate blood volume) in the pre-exercise period. The ACSM recommends consuming approximately 5 to 7 ml/kg of body weight within the 4 hours before exercise. If no urine is produced then an additional 3 to 5 ml/kg of body weight can be consumed in the 2 hours before exercise. Perhaps an easier method of achieving euhydration before exercise is by drinking adequately with meals, responding to thirst cues during the day, and hydrating well during and after exercise bouts (more on this below). Because sodium aids in fluid retention, lightly salting food or consuming drinks or foods that contain sodium will help to achieve pre-exercise euhydration as well as help to maintain sodium balance during exercise.

Hydration during Exercise

The goal of a hydration strategy during exercise should be to avoid the extremes of fluid dysregulation (i.e., dehydration or overhydration, or hyponatremia). When training, particularly for endurance events, female athletes should weigh themselves before and after exercise to determine sweat rates and, thus, fluid requirements. It may be helpful to conduct these calculations while exposed to a number of different training intensities and environmental conditions in order to determine the range of sweat rates (and fluid requirements) under a variety of circumstances. It is not necessary to replace all of the fluid lost through sweating during exercise. Rather, the goal is to avoid a fluid loss greater than 2% of body weight (Sawka et al. 2007). If this kind of assessment proves too difficult, a general guideline for drinking of 0.4 to 0.8 L/h has been recommended, with smaller women closer to the lower range. Contrary to popular sport nutrition "wisdom," thirst is an adequate indicator for the need for fluids, particularly during shorter athletic events (<2 h). However, for longer endurance events (>2 h), it is advisable for athletes to drink modestly and regularly throughout the exercise bout, because thirst may not keep up with the excessive fluid losses, and "catching up" during a race or a long training bout is likely to impair performance. Generally, beverages with 20 to 40 mmol/L of sodium are recommended. It is also advisable for the athlete to practice her competition drinking strategy—including both the amounts and types of fluids—during her training bouts. Sports drinks or snacks containing carbohydrates and electrolytes are also of value during longer races and should be introduced during training. Of course, the fluids used during training should also be used in competition (familiarity is key to minimizing gastrointestinal distress). Female athletes should either bring their own fluids to competitions, but it is much easier to inquire from race directors about the hydration products available during the race and use these drinks during their training bouts.

Post-Exercise Hydration

The goal of post-exercise fluid replacement is to replenish the fluids and electrolytes lost during exercise. If the fluid loss is extreme or if the time period before the next training bout is short, this should be accomplished as quickly as possible. Even under these conditions, the athelete should exercise caution with respect to the amount and type of fluids consumed. Large quantities of hypotonic fluids consumed post-exercise will preferentially replenish plasma volume, which will induce urination, suppress thirst, and actually slow rehydration. Adding tonicity in the form of sodium, as well as other electrolytes lost during exercise (e.g., potassium, chloride) will not only increase overall fluid retention but will also continue to stimulate thirst receptors and replace the lost electrolytes. Individuals looking to achieve rapid and complete recovery from dehydration should drink ~1.5 L of fluid per kilogram of body weight lost (Sawka et al. 2007). If there are at least 24 hours before the next training bout, then fluids can be readily replaced via drinking adequately with meals and responding to thirst cues. It is clearly more difficult to assess sodium losses during exercise, so consumption of salty foods is recommended during recovery.

SUMMARY

The important female ovarian hormones—estrogens and progesterone—have physiological effects on fluid and electrolyte regulation, primarily by shifting for the set point (to a lower osmotic level) for the regulation of thirst and fluid and sodium-regulating hormones during exercise. Nonetheless, these effects do not appear to translate into alterations in fluid or electrolyte requirements to support exercise in young, healthy women. Thus, there is no need for women athletes to change their hydration routine during different phases of their menstrual cycle or as a result of oral contraceptive use. Variability among women with respect to fitness level, body size, and acclimatization as well as the external environment play more significant roles in the thermoregulation and fluid and sodium requirements than do differences in hormonal levels and exposures. Women athletes should determine their individual sweat rates and their individual fluid and sodium requirements. Hydration strategies (including the amounts and types of fluids and electrolytes consumed) to be used in competition should be practiced during training.

REFERENCES

Aitken J M, Lindsay R, and Hart D M. The redistribution of body sodium in women on long-term oestrogen therapy. *Clin Sci Mol Med* 47: 179–187, 1974.

Almond C S D, Shin A Y, Fortescue E B, Mannix R C, Wypij D, Binstadt B A, Duncan C N, Olson D P, Salerno A E, Newburger J W, and Greenes D S. Hyponatremia among runners in the Boston Marathon. *N Engl J Med* 352: 1550–1556, 2005.

Araki T, Matsushita K, Umeno K, Tsujino A, and Toda Y. Effect of physical training on exercise-induced sweating in women. *J Appl Physiol* 51: 1526–1532, 1981.

Arieff A I. Hyponatremia, convulsions, respiratory arrest, and permanent brain damage after elective surgery in healthy women. *N Engl J Med* 314: 1529–1535, 1986.

Ayus J C, and Arieff A I. Brain damage and postoperative hyponatremia: The role of gender. *Neurology* 46 [Review]: 323–328, 1996.

Ayus J C, Varon J, and Arieff A I. Hyponatremia, cerebral edema, and noncardiogenic pulmonary edema in marathon runners. *Ann Intern Med* 132: 711–714, 2000.

Ayus J C, Wheeler J M, and Arieff A I. Postoperative hyponatremic encephalopathy in menstruent women. *An Intern Med* 117: 891–897, 1992.

Baker M A, Dawson D D, Peters C E, and Walker A M. Effects of estrogen on thermoregulatory evaporation in rats exposed to heat. *Am J Physiol* 267 (*Regulatory, Integrative, Comp Physiol* 36): R673–R677, 1994.

Barron W M, Schreiber J, and Lindheimer M D. Effect of ovarian sex steroids on osmoregulation and vasopressin secretion in the rat. *Am J Physiol* 250 (*Endocrinol Metab* 13): E352–E361, 1986.

Blahd W H, Lederer M A, and Tyler E T. Effects of oral contraceptives on body water and electrolytes. *J Reprod Med* 13: 223–225, 1974.

Calzone W L, Silva C, Keefe D L, and Stachenfeld N S. Progesterone does not alter osmotic regulation of AVP. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R2011–R2020, 2001.

Carter R, 3rd, Cheuvront S N, Williams J O, Kolka M A, Stephenson L A, Sawka M N, and Amoroso P J. Epidemiology of hospitalizations and deaths from heat illness in soldiers. *Med Sci Sports Exerc* 37: 1338–1344, 2005.

De Souza M, Maresh C, Maguire M, and Kraemer W. Menstrual cycle status and plasma vasopressin, renin activity, and aldosterone exercise responses. *J Appl Physiol* 67: 736–743, 1989.

Fortney S M, Wenger C B, Bove J R, and Nadel E R. Effects of plasma volume on forearm venous and cardiac stroke volumes during exercise. *J Appl Physiol* 55: 884–890, 1983.

Fraser C L, and Arieff A I. Epidemiology, pathophysiology, and management of hyponatremic encephalopathy. *Am J Med* 102: 67–77, 1997.

Fraser C L, Kucharczyk J, Arieff A I, Rollin C, Sarnacki P, and Norman D. Sex differences result in increased morbidity from hyponatremia in female rats. *Am J Physiol* 256 (*Regulatory, Integrative Comp Physiol* 25): R880–R885, 1989.

Fraser C L, and Sarnacki P. Na+-K+-ATPase pump function in rat brain synaptosomes is different in males and females. *Am J Physiol* 257 (*Endocrinol Metab* 20): E284–E289, 1989.

Gagnon D, Dorman L E, Jay O, Hardcastle S, and Kenny G P. Core temperature differences between males and females during intermittent exercise: Physical considerations. *Eur J Appl Physiol* 105: 453–461, 2009.

Gagnon D, Jay O, Lemire B, and Kenny G P. Sex-related differences in evaporative heat loss: The importance of metabolic heat production. *Eur J Appl Physiol* 104: 821–829, 2008.

Harrison M H. Effects of thermal stress and exercise on blood volume in humans. *Physiol Rev* 65: 149–209, 1985.

Haslag S W M, and Hertzman A B. Temperature regulation in young women. *J Appl Physiol* 20: 1283–1288, 1965.

Hirata K, Nagasaka T, Hirashita M, Takahata T, and Nuriomura T. Effects of human menstrual cycle on thermoregulatory vasodilation during exercise. *Euro J Appl Physiol* 54: 559–565, 1986.

Horvath S, and Drinkwater B. Thermoregulation and the menstrual cycle. *Aviat Space Environ Med* 53: 790–794, 1982.

Kenny G P, Leclair E, Sigal R J, Journeay W S, Kilby D, Nettlefold L, Reardon F D, and Jay O. Menstrual cycle and oral contraceptive use do not modify postexercise heat loss responses. *J Appl Physiol* 105: 1156–1165, 2008.

Kolka M, and Stephenson L. Control of sweating during the human menstrual cycle. *Eur J Appl Physiol* 58: 890–895, 1989.

Kuwahara T, Inoue Y, Abe M, Sato Y, and Kondo N. Effects of menstrual cycle and physical training on heat loss responses during dynamic exercise at moderate intensity in a temperate environment. *Am J Physiol Regul Integr Comp Physiol* 288: R1347–R1353, 2005a.

Kuwahara T, Inoue Y, Taniguchi M, Ogura Y, Ueda H, and Kondo N. Effects of physical training on heat loss responses of young women to passive heating in relation to menstrual cycle. *Eur J Appl Physiol* 94: 376–385, 2005b.

Millard-Stafford M, Sparling P B, Rosskopf L B, Snow T K, DiCarlo L J, and Hinson B T. Fluid intake in male and female runners during a 40-km field run in the heat. *J Sports Sci* 13: 257–263, 1995.

Morimoto T. Thermoregulation and body fluids: Role of blood volume and central venous pressure. *Jpn J Physiol* 40: 165–179, 1990.

Nose H, Mack G W, Shi X, Morimoto K, and Nadel E R. Effect of saline infusion during exercise on thermal and circulatory regulations. *J Appl Physiol* 69: 609–616, 1990.

Nose H, Mack G W, Shi X, and Nadel E R. Role of osmolality and plasma volume during rehydration in humans. *J Appl Physiol* 65: 325–331, 1988.

Pivarnik J, Marichal C, Spillman T, and Morrow J. Menstrual cycle phase affects temperature regulation during endurance exercise. *J Appl Physiol* 72: 542–548, 1992.

Roberts M F, Wenger C B, Stolwijk J A, and Nadel E R. Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol* 43: 133–137, 1977.

Sawka M N, Burke L M, Eichner E R, Maughan R J, Montain S J, and Stachenfeld N S. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* 39: 377–390, 2007.

Sawka M N, and Pandolf K B. Effects of body water loss on exercise performance and physiological functions. In: *Perspectives in Exercise Science and Sports Medicine. Fluid Homeostasis during Exercise*, edited by C V Gisolfi and D R Lamb. Indianapolis, IN: Benchmark, 1990, pp. 1–38.

Sawka M N, and Wenger C B. Physiological responses to acute exercise heat stress. In: *Human Performance Physiology and Environmental Medicine at Terrestrial Extremes*, edited by K B Pandolf, M N Sawka, and R R Gonzalez. Indianapolis, IN: Benchmark, 1988, pp. 97–151.

Sawka M N, Young A J, Latzka W A, Neufer P D, Quigley M D, and Pandolf K B. Human tolerance to heat strain during exercise: Influence of hydration. *J Appl Physiol* 73: 368–375, 1992.

Shapiro Y, Pandolf K B, Avellini B A, Pimental N A, and Goldman R F. Physiological responses of men and women to humid and dry heat. *J Appl Physiol* 49: 1–8, 1980.

Speedy D B, Noakes T D, and Schneider C. Exercise-associated hyponatremia: A review. *Emergency Med* 13: 17–27, 2001.

Stachenfeld N S. Acute effects of sodium ingestion on thirst and cardiovascular function. *Curr Sports Med Rep* 7: S7–S13, 2008.

Stachenfeld N S, DiPietro L, Kokoszka C A, Silva C, and Nadel E R. Physiological reliability of fluid regulation hormones in young women. *J Appl Physiol* 86: 1092–1096, 1999.

Stachenfeld N S, DiPietro L, Palter S F, and Nadel E R. Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. *Am J Physiol* 274 (*Regulatory Integrative Comp Physiol* 43): R187–R195, 1998.

Stachenfeld N S, Gleim G W, Zabetakis P M, and Nicholas J A. Fluid balance and renal response following dehydrating exercise in well-trained men and women. *Eur J Appl Physiol* 72: 468–477, 1996.

Stachenfeld N S, and Keefe D L. Estrogen effects on osmotic regulation of AVP and fluid balance. *Am J Physiol Endocrinol Metab* 283: E711–E721, 2002.

Stachenfeld N S, Silva C, and Keefe D L. Estrogen modifies the temperature effects of progesterone. *J Appl Physiol* 88: 1643–1649, 2000.

Stachenfeld N S, Silva C S, Keefe D L, Kokoszka C A, and Nadel E R. Effects of oral contraceptives on body fluid regulation. *J Appl Physiol* 87: 1016–1025, 1999.

Stachenfeld N S, Splenser A E, Calzone W L, Taylor M P, and Keefe D L. Sex differences in osmotic regulation of AVP and renal sodium handling. *J Appl Physiol* 91: 1893–1901, 2001.

Stachenfeld N S, and Taylor H S. Effects of estrogen and progesterone administration on extracellular fluid. *J Appl Physiol* 96: 1011–1018, 2004.

Stachenfeld N S, and Taylor H S. Sex hormone effects on body fluid and sodium regulation in women with and without exercise-associated hyponatremia. *J Appl Physiol* 107: 864–872, 2009.

Stachenfeld N S, Taylor H S, Leone C A, and Keefe D L. Oestrogen effects on urine concentrating response in young women. *J Physiol* 552.3: 869–880, 2003.

Stephenson L A, and Kolka M A. Esophageal temperature threshold for sweating decreases before ovulation in premenopausal women. *J Appl Physiol* 86: 22–28, 1999.

Stephenson L A, and Kolka M A. Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *Am J Physiol* 249: (*Regulatory Integrative Comp Physiol* 18): R186–R191, 1985.

Tankersley C G, Nicholas W C, Deaver D R, Mikita D, and Kenney W L. Estrogen replacement therapy in middle-aged women: Thermoregulatory responses to exercise in the heat. *J Appl Physiol* 73: 1238–1245, 1992.

Toney G M, and Stocker S D. Hyperosmotic activation of CNS sympathetic drive: Implications for cardiovascular disease. *J Physiol* 588: 3375–3384, 2011.

Tripathi A, Mack G W, and Nadel E R. Cutaneous vascular reflexes during exercise in the heat. *Med Sci Sports Exercise* 22: 796–803, 1990.

Whitten C L, and Bradbury J T. Hemodilution as a result of estrogen therapy. Estrogenic effects in the human female. *Proc Soc Exp Biol* 78: 626–629, 1951.

5 Trace Minerals of Concern for Female Athletes
Iron and Zinc

Pamela Hinton

CONTENTS

INTRODUCTION

Iron and zinc are trace minerals that are of particular concern for female athletes. Both iron and zinc are critical to athletic performance due to their essential roles in energy metabolism, gas exchange, acid and base balance, protein synthesis, and immune function. The combination of increased need for these minerals due to regular physical training and suboptimal dietary intakes puts female athletes at risk for iron and zinc insufficiency with potential adverse effects on performance.

This chapter will provide important background information on iron and zinc so as to better highlight the key roles that these two minerals play in the health and performance of the female athlete. Suggestions for helping female athletes meet their iron and zinc requirements will also be addressed.

IRON

Iron is a transition metal, and in mammalian systems iron is present in three different "redox" states: the ferrous (Fe^{+2}), ferric (Fe^{+3}), and ferryl (Fe^{+4}) redox states. Because iron exists in these different redox states, it participates in electron-transfer and oxidation-reduction reactions and reversibly binds ligands (most commonly oxygen, nitrogen, and sulfur). There are four classes of functional iron-containing proteins that facilitate these reactions: iron-containing nonenzymatic proteins (e.g., hemoglobin and myoglobin); iron-sulfur enzymes; heme-containing enzymes; and iron-containing noniron-sulfur, nonheme enzymes (Beard 2001). Many of these functional forms of iron are essential in processes that affect athletic performance, such as oxygen transfer and energy metabolism. An understanding of the metabolism of iron is important because of roles that iron plays in athletic performance as well as the high prevalence of iron deficiency among female athletes (Eliakim et al. 2002; Sinclair and Hinton 2005; Gropper et al. 2006; Woolf et al. 2009; Milic et al. 2011).

Whole-Body Iron Homeostasis

In the adult female of reproductive age, the normal iron content of the body is approximately 40 mg iron/kg of body weight. The functional iron pool accounts for approximately 75 to 90% of total body iron: 65 to 70% of total body iron is present in hemoglobin in red blood cells and 10 to 15% is in intracellular iron-containing

TABLE 5.1
Laboratory Measurements Commonly Used in the Evaluation of Iron Status

Stage of Iron Deficiency	Indicator	Diagnostic Range
Depleted stores	Stainable bone marrow iron	Absent
	Total iron binding capacity	>400 µg/dl
	Serum ferritin concentration	<12 µg/L
Early functional iron deficiency	Transferrin saturation	<16%
	Free erythrocyte protoporphyrin	>70 µg/dl erythrocyte
	Serum transferrin receptor	>8.5 mg/L
Iron-deficiency anemia	Hemoglobin concentration	<130 g/L male
		<120 g/L female
	Mean cell volume	<80 fL

Source: FNB, 2001, *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Nickel, Silicon, Vanadium, and Zinc, N. A. o., Sciences*, Washington, DC: Institute of Medicine. With permission.

proteins (i.e., myoglobin, enzymes, and cytochromes). Storage iron (i.e., iron bound to ferritin or hemosiderin) accounts for ~10 to 25% of total iron, while only a small proportion is in circulation as free iron or bound to the transport protein transferrin. Maintenance of both the functional iron pool and adequate iron stores is determined by the balance between intestinal absorption of dietary iron and iron losses via sweat, skin, urine, and gastrointestinal tract; iron is not actively excreted from the body. The high prevalence of iron deficiency among female athletes can be attributed to a combination of inadequate iron intake, compromised bioavailability, and increased iron losses.

When intake and absorption of dietary iron is inadequate to match iron losses, iron deficiency will develop, progressing through three stages: depleted iron stores (but the amount of iron in hemoglobin and other iron-containing proteins is unchanged), early functional iron deficiency without anemia, and iron-deficiency anemia (Table 5.1) (FNB 2001). In the first stage, the body will draw on the iron that is stored in the liver, spleen, or bone marrow bound to the protein ferritin. In this earliest stage of deficiency, the individual will be asymptomatic as the stored iron is adequate to maintain normal production of hemoglobin and other iron-containing proteins. Once the body has used up all of the stored iron, synthesis of iron-containing proteins, many of which are essential to athletic performance, will be negatively affected.

Dietary Iron

The Estimated Average Requirement (EAR) for iron for adult women of reproductive age (19 to 50 years) was set to maintain the functional iron pool with minimal iron stores (i.e., serum ferritin >15 µg/L) using factorial modeling of basal and menstrual iron losses and iron accretion (FNB 2001). The EAR for pregnant women accounts for basal iron losses (250 mg for the entire pregnancy), iron deposited in the fetus and placenta (320 mg), net iron lost in the expansion of maternal red blood cell mass

TABLE 5.2
Iron and Zinc Estimated Average Requirements (EARs) and Recommended Dietary Allowances (RDAs) for Adult Women

	Iron (mg)		Zinc (mg)	
	EAR	RDA	EAR	RDA
Nonpregnant, nonlactating				
19–30 years	8.1	18	6.8	8
31–50 years	8.1	18	6.8	8
51–70 years	5	8	6.8	8
>70 years	5	8	6.8	8
Pregnant				
19–30 years	22	27	9.5	11
31–50 years	22	27	9.5	11
Lactating				
19–30 years	6.5	9	10.4	12
30–50 years	6.5	9	10.4	12

minus iron (300 mg), and an increase in iron bioavailability from 18 to 25% in the second and third trimesters. For nonmenstruating, lactating women, the EAR is derived using basal iron losses plus the iron secreted into human milk (0.35 mg Fe/L human milk × 0.78 L milk/day). The EARs and Recommended Dietary Allowances (RDAs) for adult women are shown in Table 5.2. Iron requirements are increased during adolescence to meet the demands of growth and inevitable losses. Although female athletes are at increased risk for iron deficiency because of increased iron losses, the RDA for physically active women does not differ from their less-active peers.

The iron content of the typical American diet is approximately 6 mg iron/1000 kcal, of which 10 to 15% is heme iron (from meat) and 85 to 90% is nonheme iron (from meat and plant sources) (Beard and Tobin 2000). The bioavailability of iron, which is the amount of iron present in food which is absorbed, is lower for nonheme iron (~5% of nonheme iron is absorbed from a vegan diet and ~10% for a mixed diet) than for heme iron (~25% of heme iron is absorbed). Because of the lower bioavailability of nonheme iron compared with heme iron, vegetarians have an iron requirement that is 1.8 times that of individuals who consume a mixed diet (FNB 2001). The low bioavailability of nonheme iron is partially due to its low solubility even in the acidic environment of the stomach. In addition, absorption of nonheme iron can be reduced up to twofold by co-ingestion with other nutrients or dietary compounds. For example, the absorption of nonheme iron is inhibited in the presence of phytates (found in nuts, seeds, legumes, and whole grains), and polyphenols, which are present in tea and coffee. The bioavailability of nonheme iron also is reduced in the presence of other minerals such as zinc and calcium. Conversely, other dietary components increase the bioavailability of nonheme iron. For example, organic acids

such as ascorbic acid (vitamin C) and citric acid enhance nonheme iron absorption. An unidentified compound (or compounds), probably short peptides, in meat also enhances absorption of nonheme iron. The estimated overall bioavailability of iron from a typical American diet is 18% (FNB 2001).

Iron Absorption

Whole-body iron content is regulated at the level of intestinal absorption because iron is not actively excreted by the body. Nonheme dietary iron is found in the ferric (Fe^{+3}) redox state but must be reduced to the ferrous form prior to absorption. Ferrireductase (Dcytb) of the duodenal mucosa reduces ferric iron to ferrous iron, which can then be transported across the mucosal membrane via divalent metal transporter-1 (DMT-1) (McKie et al. 2001). Absorption of heme iron occurs via a mechanism independent of that for nonheme iron. Heme iron binds a receptor, is internalized, and then is degraded within the enterocyte releasing iron. Iron transfer across the basolateral membrane occurs via the iron-transport protein ferroportin 1. Iron that is not exported from enterocytes is lost from the body when the mucosal cells are sloughed every 48 to 72 hours. Both iron uptake at the mucosal membrane and iron release at the basolateral membrane are regulated by iron status. Iron uptake and release are both downregulated in response to adequate iron status. Conversely, when there is a demand for increased body iron (e.g., elevated erythropoiesis), iron absorption and export are increased via hormonally mediated changes in DMT-1 and ferroportin 1 expression. Recently, altered iron export via ferroportin 1 has been identified as a possible contributor to exercise-associated iron deficiency (Peeling et al. 2008).

Iron Losses

Daily basal iron losses (0.9 to 1.2 mg iron/day) are small compared to total body iron content: 0.6 mg/day are lost from the gastrointestinal tract, mostly in sloughed mucosal cells; 0.08 mg/day are lost in urine; and 0.2 to 0.3 mg/day are lost in skin. Menstruating women require an additional 0.6 to 0.9 mg/day to account for menstrual blood loss. Women with heavy menstrual blood flow and women who use intrauterine devices will have greater iron losses. Because oral contraceptive agents tend to reduce menstrual bleeding (FNB 2001), their use is associated with reduced iron loss.

Physical activity increases the amount of iron lost via sweat, urine, and the gastrointestinal tract. Exercise-associated blood and therefore iron loss are greatest after high-intensity, prolonged, or dynamic, weight-bearing activities. Thus, regular weight-bearing exercise of moderate to high intensity can increase iron losses by 30 to 70% (FNB 2001). The amount of iron lost in sweat is increased simply because physically active individuals sweat more than their sedentary counterparts. Although sweat volume is increased during exercise in hot environments, the concentration of iron in sweat declines with heat acclimation to conserve iron (Chinevere et al. 2008). Exercise may cause hematuria (blood in urine). Reasons for elevated urinary blood losses are damage to the kidney because of lack of oxygen, increased renal blood pressure, increased body temperature, exercise-associated acidosis, renal or bladder trauma, and dehydration. Similarly, reduced blood flow, increased gut motility, and mechanical trauma, resulting from exercise, increase gastrointestinal blood

losses. Iron losses are increased due to shortened red blood cell (RBC) life span, which can be reduced significantly (e.g., 74 days in runners versus 141 days in sedentary controls) (Weight, Darge et al. 1991). The increased rate of hemolysis results from general circulatory (i.e., mechanical) trauma, oxidative damage due to elevated production of superoxide, or osmotic changes that induce changes in RBC volume, increasing fragility. Exercise-induced hemolysis has been associated with swimming (Selby and Eichner 1986), cycling, rowing (Telford et al. 2003), and strength training (Schobersberger et al. 1990), and appears to be positively related to exercise intensity (McInnis et al. 1998). However, the primary determinant of hemolysis is "footstrike" (Telford et al. 2003). During activities that involve running or jumping, red blood cells in the capillaries of the bottom of the foot are destroyed by the mechanical forces experienced upon impact with the ground and hence the term *footstrike hemolysis*. These mechanisms may explain why both exercise duration and intensity are negatively associated with hemoglobin, hematocrit, and serum ferritin concentrations in highly trained athletes (Schumacher et al. 2002a; Wilkinson et al. 2002). Chronic use of aspirin or nonsteroidal anti-inflammatory drugs may augment exercise-associated blood (i.e., iron) losses by increasing clotting time.

Iron Recycling

The amount of iron absorbed by the small intestine daily (~1 to 2 mg) is insufficient both to replace daily losses (~1 mg/day) and to meet the demands of erythropoiesis (~22 mg/day). Iron that is salvaged from old or damaged red blood cells by the macrophages in the liver and spleen amounts to ~22 mg/day, thus supplying most of the iron taken up by the bone marrow each day for the production of RBCs (Anderson et al. 2007).

Export of iron from macrophages, enterocytes, and hepatocytes via the iron transport protein ferroportin 1 is highly regulated, as iron released from these cells controls iron availability to the rest of the body (Anderson et al. 2007). The primary regulator of iron export is the peptide hormone hepcidin, which is produced by hepatocytes. Hepcidin lowers iron efflux by reducing the amount of ferroportin 1 available for iron export. Hepcidin production is regulated by hepatic iron status, erythropoiesis, hypoxia, and inflammation (Leong and Lonnerdal 2004). The increase in hepcidin in response to inflammatory proteins (e.g., interleukin-6, IL-6), is thought to serve an antimicrobial function by reducing iron available to bacteria.

Recently, exercise-induced inflammation has been implicated as a possible cause of iron deficiency in athletes (Peeling 2008). Anemia associated with chronic inflammation is due to sequestration of iron as a result of increased hepcidin (Nemeth et al. 2003). Roecker et al. (2005) was the first to report that hepicidin is increased following exercise, noting that urinary hepcidin was increased 24 hours after a marathon in 8 of the 14 female runners studied (Roecker et al. 2005). More recently, Peeling et al. (2009) found that IL-6, free hemoglobin, and hepcidin were increased by high-intensity running in highly trained male runners (Peeling et al. 2009a,b). Although exercise stimulates IL-6 release from exercising skeletal muscle (Toft et al. 2010) in a mode-, intensity-, and duration-dependent manner (Fischer 2006), exercise duration is the strongest determinant of IL-6 release (Fischer 2006). For example, relative to

pre-exercise concentrations, IL-6 is increased 16-fold after 2 hours, 100-fold after 6 hours, and 10,000-fold after a 36-hour ultra-endurance footrace (Margeli et al. 2005; Fischer 2006). Exercise-associated IL-6 release is also increased when skeletal muscle glycogen is depleted (Steensberg 2001) and following exercise in hot environments (Fischer 2006). Although more data are needed to establish a causal role for chronic inflammation and hepcidin in the iron deficiency associated with sports participation, particularly endurance sports, it is likely to play a role.

Iron and Athletic Performance

Iron plays a critical role in oxidative metabolism and thus is especially important for the female endurance athlete whose success depends on a high aerobic capacity. Hemoglobin and myoglobin bind oxygen via the porphyrin ring of heme. Hemoglobin in RBCs carries oxygen from the lungs to the exercising skeletal muscles; myoglobin transfers oxygen from erythrocytes to muscle cells. The electron transport chain, which is the final step in oxidative ATP synthesis, depends on heme-containing cytochromes (a, a_3, b, b_5, c, c_1) and on nonheme iron-sulfur enzymes (NADH dehydrogenase, succinate dehydrogenase, and ubiquinone-cytochrome c reductase) (Beard and Tobin 2000). Thus, iron deficiency impairs ATP production and increases reliance on anaerobic metabolism of glucose to produce ATP, in effect reducing endurance capacity. Given the essential roles of these iron-containing proteins in both oxygen utilization and aerobic metabolism, iron status has the potential to affect both maximal and submaximal exercise capacity.

Maximal Aerobic Capacity

The effects of iron status on aerobic capacity have been demonstrated by comparison of iron-deficient animals (or humans) to those with normal iron status. Animals with experimentally induced iron deficiency show marked reductions (~30 to 70%) in hemoglobin, myoglobin, muscle mitochondria content, and in mitochondrial iron-dependent proteins and reduced maximal oxygen consumption VO_{2max} (Davies et al. 1982). In addition, animals with the most severe anemia (i.e., lowest hemoglobin concentrations) have the lowest VO_{2max} (Perkkio et al. 1985). Humans with experimentally induced anemia also exhibit reductions in VO_{2max}, which are proportional to hemoglobin concentrations (Woodson et al. 1978; Celsing et al. 1986).

Additional evidence that iron status affects maximal aerobic capacity comes from supplementation trials in which anemic subjects are iron repleted (i.e., brought back up to normal iron status) via supplementation and aerobic capacity is measured before and after repletion. For example, in one of the earliest supplementation trials to examine the effects of iron deficiency on physical performance, Gardner et al. (1977) showed that iron supplementation of anemic women improved iron status and performance during a standardized, multistage treadmill test work capacity and reduced exercise heart rate and blood lactate concentrations. It is important to note that iron supplementation of iron-deficient nonanemic subjects (i.e., normal hemoglobin) does not improve VO_{2max} (Rowland et al. 1988; Newhouse et al. 1989; Klingshirn et al. 1992; Zhu and Haas 1998a), as the primary determinant of VO_{2max} is oxygen delivery.

Submaximal Aerobic Capacity

To demonstrate the role of iron in aerobic metabolism during submaximal exercise, animals with experimentally induced anemia are transfused with RBCs to normalize hemoglobin concentrations, while other iron-containing proteins remain reduced. Maximal oxygen consumption is restored after correction of hemoglobin, but endurance during submaximal aerobic exercise remains impaired (Finch et al. 1976). The results of iron supplementation studies of women who are iron depleted but not anemic also suggest that iron affects aerobic metabolism during submaximal work (Rowland et al. 1988). From an athletic performance perspective, diminished submaximal exercise capacity translates into reduced endurance, which is defined as the ability to sustain a submaximal workload until exhaustion.

Accurate measurement of endurance in humans is difficult because study participants must exercise for extended periods of time to achieve exhaustion, and performance is highly dependent on subject motivation. One strategy to measure endurance capacity in human subjects is to use a time trial of sufficient distance that subjects exercise at a submaximal intensity for the majority of the test, yet are motivated by a defined end point. Zhu and Haas (1998a) found that iron supplementation reduced energy expenditure and fractional utilization of peak oxygen consumption in iron-depleted nonanemic women. Likewise, Hinton et al. (2000) found that iron supplementation during 4 weeks of aerobic exercise training resulted in greater improvements in endurance compared with placebo (Hinton et al. 2000) and the effect of the iron supplement was greatest in subjects with the greatest potential to respond (i.e., elevated tissue iron need as defined by serum transferrin receptor [sTfR >8 mg/L] at baseline) (Brownlie et al. 2004). Using a progressive muscle fatigue protocol to assess submaximal work capacity, Brutsaert et al. (2003) found that iron supplementation of iron-deficient nonanemic women attenuated onset of fatigue in maximal voluntary contractions.

Dietary Iron Intakes in Female Athletes

Comparison of studies that report the prevalence of suboptimal iron intake in female athletes is complicated by the evolution of recommended nutrient intakes over time and by differences in how investigators ascertained adequacy and define adequacy. With the current Dietary Reference Intakes (DRIs), the EAR is the appropriate standard to evaluate nutrient intakes of groups (FNB 2000); however, the EAR for athletes may be increased by 30 to 70% (FNB 2001) due to elevated iron losses. Studies that were conducted prior to the establishment of the DRIs for iron in 2001 often used two-thirds of the former RDA, which was 15 mg/day (FNB 1989), as the definition of sufficient dietary iron intake. There is evidence that adolescent and collegiate female athletes in a wide variety of sports do not consume enough dietary iron (Beals 2002; Kim et al. 2002; Papadopoulou et al. 2002; Ziegler et al. 2002; Gropper et al. 2006); although others have reported that most female athletes consume adequate dietary iron (Steen et al. 1995; Hinton et al. 2004). However, as mentioned above, these discrepant results may be partially explained by differences in the value used to define adequacy—that is, use of the RDA (18 mg/day) versus the EAR (8 mg/day).

Iron Supplementation in Female Endurance Athletes

Few studies have assessed the effects of iron supplementation on endurance performance in female athletes. McClung et al. (2009) reported that iron supplementation prevented the decline in iron stores following 8 weeks of basic combat training and enhanced training adaptations as assessed by 2-mile run times in female soldiers. Moreover, the benefits of iron supplementation were greatest in subjects with iron-deficiency anemia (McClung et al. 2009). Similarly, Hinton and Sinclair (2007) found that iron supplementation of iron-deficient nonanemic women positively affected ventilatory threshold (VT) and gross energetic efficiency during a steady-state submaximal test in chronically trained (≥60 min/day; ≥3 days/week; ≥6 month) subjects (17 women, 3 men) versus placebo. The effects of iron supplementation on VT were greatest in participants with the greatest potential to respond (i.e., lowest presupplementation ferritin). Moreover, increases in serum ferritin were associated with reductions in respiratory exchange ratio (increased fatty acid versus glucose oxidation) and VT (% VO_{2peak}) during steady-state submaximal (60% VO_{2peak}) exercise. Thus, it appears that even marginal repletion of iron stores in iron-depleted trained women positively affects aerobic function (Hinton and Sinclair 2007).

Iron Deficiency

As discussed above, early functional iron deficiency reduces endurance capacity and energetic efficiency during submaximal exercise in young women (Hinton et al. 2000; Brownlie et al. 2004; Hinton and Sinclair 2007). Based on animal studies, this deficit is due to decreased activity of iron-containing oxidative enzymes and cytochromes (Finch et al. 1976; Davies et al. 1982; Willis et al. 1987). The hallmark symptoms of iron-deficiency anemia are fatigue, lack of energy, and apathy. Specifically, anemia impairs maximal exercise performance (maximal oxygen consumption, VO_{2max}) by reducing oxygen delivery to the body (Celsing et al. 1986).

In addition to performance-related deficits, iron deficiency has other negative consequences that impact the overall health of female athletes. Iron-deficiency anemia impairs nonspecific immunity by decreasing the ability of macrophages and neutrophils to kill pathogens (Beard 2001). Anemia may play a causal role in "restless legs syndrome" (Earley et al. 2000). Iron-deficiency anemia also has been associated with postpartum depression and impaired cognition in women of low (Beard et al. 2005) and high (Corwin et al. 2003) socioeconomic status. A recent study reported that severe iron deficiency was associated with reduced activity of the high-density lipoprotein (HDL)–associated enzyme paraoxonase, which protects lipoproteins against oxidative damage by neutralizing lipid hydroperoxides (Martinovic).

Prevalence of Iron Deficiency

In the general population of adults in the United States, 3 to 5% of women are anemic and 11 to 13% are iron deficient (Looker et al. 1997). The reasons for the relatively high prevalence of iron deficiency in women are increased iron losses in menstrual blood flow and lower dietary iron intake. In female athletes, exercise-associated losses (sweat, footstrike hemolysis, gastrointestinal, etc.) may also contribute to the high prevalence.

The prevalence of iron deficiency, particularly nonanemic iron depletion (i.e., normal hemoglobin and hematocrit, but low serum ferritin and elevated sTfR), appears to be greater in physically active women and competitive female athletes compared with their sedentary counterparts (Eliakim et al. 2002; Fallon 2004; Gropper et al. 2006; Woolf et al. 2009; Milic et al. 2011). As with the assessment of dietary intake, comparison of studies that report prevalence of iron depletion is complicated by use of different cutoff values for the iron status indicators (i.e., ferritin ranging from 12 to 30 ng/ml has been used as the criterion for iron depletion). Nevertheless, the prevalence of iron depletion appears to be ~20 to 30% in female athletes with higher frequency in endurance athletes (e.g., distance runners and triathletes) (Rietjens et al. 2002; Lukaski 2004; Sinclair and Hinton 2005).

Groups at Risk for Iron Deficiency

In addition to suboptimal iron intakes and increased physical activity–associated losses, athletes who are frequent blood donors are also at increased risk for iron deficiency (Choe et al. 2001), as 200 to 250 mg of iron are lost per 0.5 L of blood. *Helicobacter pylori* and gastrointestinal parasitic infections also increase blood losses via the gut. In addition, multiparous women are at greater risk for iron deficiency due to pregnancy-associated depletion of iron stores. Women with heavy menstrual bleed or who use an intrauterine device for contraception are at greater risk for iron deficiency due to increased blood losses. As discussed above, athletes who follow a vegetarian or vegan diet are at higher risk for iron deficiency due to the reduced iron bioavailability in a plant-based diet. In addition, athletes who regularly use nonsteroidal anti-inflammatories are likely to have increased gastrointestinal blood losses (Rudzki et al. 1995), increasing their risk of iron deficiency. Recent data suggest that iron status may be determined, in part, by genetics (Tanaka et al. 2010).

Assessment of Iron Status

Anemia and Nonanemic Iron Depletion

Blood tests are needed to diagnose the stage of iron deficiency, including the most severe stage—iron-deficiency anemia. The concentration of hemoglobin in blood is one of the primary criteria for diagnosis of anemia. Hematocrit, the proportion of blood volume that is red blood cells, is reduced during anemia. The threshold hemoglobin and hematocrit values used to define anemia are increased in individuals who live at altitude and in cigarette smokers. Iron is transported in the blood bound to a protein called transferrin. The percent of transferrin that is carrying iron—that is, transferrin saturation—is decreased with iron deficiency.

Anemia caused by iron deficiency can be distinguished from other nutritional anemias based on the appearance of the RBCs. Iron deficiency results in insufficient hemoglobin production. The body attempts to make up for the decreased oxygen-carrying capacity by producing new red blood cells at a rapid rate. Under the microscope, these red blood cells appear small (because they are immature) and very pale (due to lack of hemoglobin). This type of anemia is called *hypochromic microcytic anemia*. In contrast, anemia caused by inadequate B_{12} or folate is due to impaired cell

division. To compensate, the existing red blood cells grow very large with normal amounts of hemoglobin and, therefore, normal pigmentation. This type of anemia is called *megaloblastic* or *macrocytic*. Mean corpuscular volume (*corpuscle* is another term for blood cell) is the clinical measure of red blood cell size. Mean corpuscular hemoglobin concentration indicates the amount of hemoglobin per red blood cell.

Depletion of iron stores is assessed by determination of serum ferritin concentrations. Because the ferritin concentration in blood is proportional to ferritin stored in the liver (1 µg ferritin/L is equivalent to ~10 mg of stored iron), serum ferritin is used as an indicator of iron stores (Cook 1979). However, ferritin is an acute phase protein, so its production in the liver is elevated during illness or inflammation, independent of iron stores. Therefore, ferritin should not be measured during illness or after an exhaustive exercise bout to avoid masking depleted iron stores. The concentration of soluble transferrin receptor (sTfR) in blood is used as an indicator of tissue iron status (Punnonen et al. 1997) because cellular expression of sTfR increases with iron need (Skikne et al. 1990). When tissues such as skeletal muscle or bone marrow need iron, the number of transferrin receptors on the cell surface increases to increase iron uptake. The use of sTfR to evaluate iron status in athletes has been advocated because sTfR is not an acute phase protein and therefore is not likely to be acutely affected by exercise. The ratio of [sTfr]/log [ferritin] also has been used to evaluate bone marrow iron depletion (Punnonen et al. 1997) with higher ratios indicating more severe iron depletion (Suominen 1998). For example, a ratio greater than 1.8 is indicative of depleted iron stores, while a ratio greater than 2.2 suggests iron-deficient erythropoiesis. The ratio is particularly useful for evaluation of iron status in athletes whose ferritin stores are depleted (i.e., <12 ng/ml) (Punnonen et al. 1997). However, because of the large day-to-day variability in the ratio for athletic populations who are actively training, only changes ≥0.4 should be considered significant (Stupnicki et al. 2003). Serum ferritin, sTfR, and [sTfr]/log [ferritin] respond to 8 weeks of iron supplementation in nonanemic iron-depleted women (Zhu and Haas 1998b).

Special Considerations for Iron-Status Assessment in Athletic Populations

The International Olympic Committee (2009) recommends that assessment of iron status, including anemia and iron stores, be part of periodic health evaluations of both male and female athletes due to the increased prevalence of iron deficiency in athletic populations. In one survey, only 43% of Division-IA National Collegiate Athletic Association (D-IA NCAA) athletic programs performed routine screening for iron deficiency (Cowell et al. 2003). Although screening large numbers of athletes makes it difficult to standardize measurement conditions, it is important to recognize that factors such as time of day, interval since last training bout, illness, or injury might impact the results and validity of the tests. Acute changes in plasma volume can mask true changes in indicators of iron status (Schumacher et al. 2002b). Endurance training can cause expansion of plasma volume that might exceed or precede the adaptive increase in RBC number, which can result in an apparently low hematocrit or hemoglobin (Weight, Darge et al. 1991). This phenomenon has been termed *dilutional anemia* or *sports anemia* but does not adversely affect performance. In addition to indicators of anemia, iron stores and tissue iron status,

reduced serum haptoglobin, hematuria, and hemoglobinuria are evidence of elevated hemolysis.

Prevention and Treatment of Iron Deficiency

Consuming an Iron-Adequate Diet

Primary prevention of iron deficiency among female athletes should focus on dietary strategies to increase consumption of iron-rich foods and foods that enhance iron absorption. Because the amount of iron in the U.S. food supply is ~6 mg per 1000 calories, achieving the recommended intake of iron (18 mg/day) through diet alone is difficult for most women. A woman would have to consume ~3000 calories to get 18 mg of iron, and this energy intake exceeds the needs and actual intakes of many female athletes. Thus, consumption of iron-rich foods, such as meat, iron-fortified grains and breakfast cereals, dried fruit, nuts, and soy nuts, is particularly important. Some "energy bars" are fortified with iron and may be significant contributors to total iron intake. Female athletes should be provided information on how to select foods that have high iron bioavailability. As previously mentioned, nonheme sources of iron have a significantly lower bioavailability than heme sources. Thus, individuals who follow a vegetarian or vegan diet must be especially careful to consume enough iron.

Iron Supplementation

Iron supplementation is warranted in athletes with diagnosed iron-deficiency anemia or serum ferritin <20 ng/ml (Pitsis et al. 2004). For treatment of iron-deficiency anemia, the Centers for Disease Control and Prevention (CDC) recommends an oral dose of 60 to 120 mg elemental iron per day as two 50 to 60 mg doses. Individuals being treated with supplemental iron should be retested after 4 weeks of supplementation, and if hemoglobin responds to treatment, supplementation should be continued for an additional 2 to 3 months to replete iron stores. Individuals of African, Mediterranean, or Southeast Asian descent who do not respond to supplemental iron should be tested for thalassemia or sickle cell trait. Anemia that does not respond to iron treatment might be due to either vitamin B_{12} or folate deficiency. Supplemental ferrous iron is available in a variety of physical and chemical forms. The most common chemical form of iron supplement is ferrous iron complexed with sulfate, succinate, citrate, lactate, fumarate, or gluconate. These preparations typically contain 35 to 100 mg of elemental iron (Beard and Tobin 2000). In addition to pills, iron is available in chewable, enteric-coated, extended-release, and liquid forms. Although the enteric-coated and extended-release forms have fewer side effects, their absorption is poor. Consequently, these forms should be avoided if possible. Under extremely rare circumstances, iron can be administered parenterally (i.e., intravenously) by a physician. Supplemental iron should be taken with ascorbic acid to enhance absorption. The bioavailability of iron from multimineral supplements, in particular those that contain calcium, is less than that of iron salts (Hallberg et al. 1992). Likewise, milk, coffee, tea, phosphate-containing soft drinks, and some medications (e.g., tetracycline, antacids, and acid blockers) reduce iron absorption and should not be consumed with the iron supplement. Gastrointestinal side effects, such as nausea,

diarrhea, constipation, and cramps, are common with iron supplementation, and athletes should be counseled on the benefits of treatment despite the potential side effects to increase compliance. Gradually increasing the dose of supplemental iron or taking the iron supplement with food might reduce the severity of the side effects. In addition, the supplement can be taken less than daily (i.e., every 2 to 3 days) to minimize side effects without negatively impacting treatment. Because iron is toxic, supplements should be used only under medical supervision. Nonetheless, studies of collegiate female athletes report that ~8% use supplemental iron, while 36% take a multivitamin with iron (Froiland et al. 2004; Herbold et al. 2004). The prevalence of iron supplementation appears higher in Olympic athletes, ranging from 12 to 21% (Huang et al. 2006).

Iron Overload

The upper intake level (UL) for iron, which applies to individuals of normal iron status, is 45 mg/day (FNB 2001). Iron overload (serum ferritin >200 ng/ml) is a real risk associated with unnecessary or unmonitored iron supplementation. Mettler and Zimmermann (2010) reported that 15% of male and 5% of female marathon runners suffered from iron overload. When body iron exceeds the storage and transport capacity of ferritin and transferrin, respectively, the excess iron remains unbound to proteins. This free iron causes lipid peroxidation and free radical production, processes that damage the cardiovascular system, kidneys, liver, and central nervous system. Moreover, genetic disorders that affect iron metabolism can increase risk of iron overload. Individuals with hereditary hemochromatosis, an autosomal recessive disorder, suffer from excessive iron absorption and impaired storage, which cause extensive organ damage. Thalassemia or sideroblastic anemia also increase risk for iron overload due to increased iron absorption, secondary to elevated erythropoiesis.

Because of the potential for iron overload, iron supplementation to correct a deficiency should be under the supervision of a healthcare professional. Athletes who experience symptoms that are consistent with iron deficiency (unusual fatigue or decrements in performance, cold intolerance, frequent illnesses) should have their iron status assessed before beginning a supplemental regimen. Once iron deficiency is identified and supplementation is initiated (e.g., 65 mg elemental iron/day), the athlete should have her iron status monitored at regular intervals (e.g., every 4 to 6 weeks).

ZINC

Introduction

Zinc has three primary functions: catalytic activity of enzymes; stabilization of proteins, including enzymes, and cell membranes; and regulation of various cellular processes such as gene expression, enzyme activity, apoptosis, hormone synthesis, and cell signaling (McCall 2000). Of particular relevance to female athletes is the fact that the metabolism of carbohydrate, protein, and fat requires numerous zinc-dependent enzymes. For example, zinc appears to activate glycolysis via its stimulatory effects on pyruvate kinase and phosphofructokinase (Brand and Kleineke 1996). Similarly, lactate dehydrogenase, which is responsible for conversion of lactate into

pyruvate, is also zinc dependent (Cordova and Alvarez-Mon 1995; Lukaski 2006). In addition to energy production, zinc is essential for other cellular processes involved in the body's response to exercise. Synthesis of nucleic acids and protein and, therefore, cell growth, also rely on zinc-containing enzymes. Superoxide dismutase, which protects membranes against oxidative damage by converting superoxide anion into water and hydrogen peroxide, requires zinc. Carbonic anhydrase, which converts carbon dioxide and water into bicarbonate ion and thus plays an important role in acid/base balance and gas exchange, is a zinc-containing enzyme. In addition, zinc is required for the synthesis and activity of many hormones that respond to physical activity and mediate the effects of exercise, including insulin, leptin, growth hormone, glucocorticoids, thyroid hormone, gonadotropins, and sex steroids (Vallee and Falchuk 1993; Lukaski 2006; Casimiro-Lopes et al. 2009).

Whole-Body Zinc Homeostasis

Nearly all zinc in the body is intracellular. Skeletal muscle and bone account for ~55% and 29% of total body zinc, respectively, but other tissues, such as the eye and hair, have higher zinc concentrations. Slow-twitch, oxidative skeletal muscle fibers (i.e., type I) contain more zinc than fast-twitch glycolytic fibers. Absorbed zinc is rapidly released from the liver and distributed to the rest of the body, constituting a rapid-turnover pool of zinc with a half-life of ~12.5 days in humans. However, the turnover of zinc in pancreas, liver, spleen, RBCs, muscle, and bone is much slower (average 300 days) with RBC, muscle, and bone having the slowest rates (Lukaski 2006).

Dietary Sources of Zinc

The EAR for zinc for adult women of reproductive age (19 to 50 years) was set to balance total daily excretion of endogenous zinc, using a factorial approach (FNB 2001). The EAR for pregnant women accounts for basal iron losses and zinc deposited in the fetus, placenta, and maternal tissues. For nonmenstruating, lactating women, the EAR is derived using basal iron losses plus the iron secreted into human milk (FNB 2001). The EARs and RDAs for adult women are shown in Table 5.2. Zinc requirements increase during adolescence to meet the demands of growth and inevitable losses. The recommended zinc intake is not increased in physically active women relative to their sedentary peers.

In the typical American diet, animal-based foods are the primary sources of dietary zinc. Meat has the highest zinc content per serving, followed by fish and dairy products. The zinc content of cereals, grains, and legumes is highly variable depending on geographic location and year. In addition, food processing and preparation methods have a significant impact on zinc content of foods. Refinement of cereals and grains can reduce the zinc content by up to 80%. Many factors affect the bioavailability of zinc, which is the amount of zinc present in food which is absorbed. Nutrients or dietary factors that increase zinc absorption are protein, in particular animal-based proteins, and glucose. By contrast, fiber and phytic acid can significantly reduce zinc absorption by as much as 50%. In addition, nonheme iron and calcium reduce zinc bioavailability (Hunt 2003).

Intestinal Absorption of Dietary Zinc

Whole-body zinc content is controlled both by regulation of zinc absorption from the small intestine and excretion via the gastrointestinal tract. Absorption of dietary zinc and endogenous zinc from pancreatic secretions occurs in the small intestine by both carrier-mediated absorption and diffusion. Zinc in food is bound to proteins or nucleic acids and must be hydrolyzed by gastric acid and enzymes prior to absorption. Once in the mucosal cell, zinc is bound to metallothionein and is either excreted into the intestinal lumen or released into the portal circulation via a zinc transport protein, depending on zinc needs (Vallee and Falchuk 1993).

Although fractional absorption of dietary zinc is inversely proportional to zinc intake (Wada et al. 1985), total zinc absorption is greater with zinc-adequate diets compared with low-zinc diets. For example, Chung et al. (2008) found that fractional zinc absorption was significantly greater (60% versus 36%) with low-zinc versus adequate-zinc meals (4 mg Zn/day versus 11 mg Zn/day), while total zinc absorbed was greater with the adequate-zinc meals. Zinc absorption is enhanced in the presence of glucose and by complex formation with amino acids, in particular histidine (Lukaski 2006). By contrast, zinc absorption is inhibited by iron and calcium and by binding to phytate or oxalic acid, which results in the formation of insoluble complexes. Polyphenols, such as tannins, which are present in coffee and tea, also inhibit zinc absorption (FNB 2001). Thus, zinc absorption will be greatest from low-phytate, zinc-adequate meals.

Basal Zinc Losses

Whole-body zinc content is determined by the balance between zinc absorption and zinc losses, including active excretion of zinc via the gastrointestinal tract and urine. Basal zinc losses include urinary losses (0.4 to 0.8 mg/day), which are relatively insensitive to zinc intake. Zinc lost via sloughed skin cells, sweat, and hair (~1 mg/day) increases in proportion to dietary zinc consumption. In addition, up to 0.5 mg Zn is lost per menstrual cycle (FNB 2001). Control of zinc excretion via the gastrointestinal tract (i.e., feces) contributes to regulation of whole-body zinc content. In individuals with typical zinc intakes, ~90% of dietary zinc is lost in feces. Although a large fraction of the zinc present in endogenous secretions (2 to 5 mg Zn/day) is reabsorbed, the amount that is lost via the feces varies with dietary intake from ~1 mg to 5 mg Zn/day at very low and very high intakes, respectively (Lukaski 2006).

Exercise appears to increase zinc losses in sweat and urine (Cordova and Alvarez-Mon 1995), although the contribution to total loss via these routes remains somewhat controversial. For example, the amount of zinc lost in sweat is relatively small, and as was also observed for iron, the concentration of zinc in sweat declines with heat acclimation to conserve zinc (Chinevere et al. 2008). Urinary zinc losses are increased acutely but return to pre-exercise values within 24 hours following exercise. The increase in urinary zinc might be due to skeletal muscle damage (Cordova and Alvarez-Mon 1995). It is generally accepted that training does not have adverse effects on zinc status if dietary intake is adequate (Lukaski 1989; Lukaski et al. 1990).

Zinc and Athletic Performance

The physiological functions of zinc have been largely identified in studies of animals with experimentally induced zinc deficiency and clinical observation of zinc-deficient humans. Zinc affects growth and development, reproductive function, taste, vision, neurotransmission and brain function, skin integrity, wound healing, immune function, gastrointestinal mucosa, platelet hemostasis, basal metabolic rate and protein utilization, and red blood cell membrane stabilization (Wada and King 1986; Vallee and Falchuk 1993; Cordova and Alvarez-Mon 1995; Lukaski 2004). Due to its role as a cofactor for enzymes involved in energy metabolism, gas exchange, and protein synthesis, zinc deficiency has the potential to negatively impact athletic performance.

Research examining zinc nutriture and athletic performance is hindered by the inability to accurately assess zinc status (see section entitled "Assessment of Zinc Status" below). Nonetheless, some limited evidence from both animal models and in humans suggests that reductions in serum zinc are associated with biochemical and physiological changes that could negatively impact performance. For example, a study in growing rats showed that zinc deficiency resulted in reduced lactate dehydrogenase activity in heart and skeletal muscle (Hambidge et al. 1986). Brun et al. (1995) reported a significant positive correlation between serum zinc and isometric muscle strength in male and female adolescent gymnasts. In a cross-sectional study of male soccer players, Khaled et al. (1997) reported that those with low serum zinc had significantly lower average power output and a greater increase in blood lactate during a VO_{2max} test on a cycle ergometer. Van Loan et al. (1999) reported that zinc depletion via a low-zinc diet (0.3 mg Zn/day for ~5 weeks) reduced total work capacity of the knee extensors and shoulder extensors and flexors in young adult males. In a double-blind, randomized crossover study, Lukaski (2005) found that 9 weeks of low zinc intake (3.8 mg/day) reduced VO_{2peak}, VCO_{2peak}, respiratory exchange ratio, and total and RBC carbonic anhydrase activity in association with reductions in serum and RBC zinc concentrations relative to adequate zinc intake (18.7 mg/day). Zinc-deficient rats exhibited reduced muscle growth and DNA content and a reduction in type IIB fibers, as well as increased muscle fatigue (Park et al. 1986). Several reports of hypozincemia in athletes suggested that training might adversely affect zinc status (Lukaski 2006). However, subsequent studies of both male and female athletes indicate that regular exercise does not impair zinc status if dietary intake is adequate (Lukaski 1989; Lukaski et al. 1990).

Studies in which subjects are supplemented with zinc also seem to support an association between zinc status and performance (Krotkiewski et al. 1982). In a placebo-controlled crossover study, 2 weeks of supplemental zinc (135 mg Zn/day) increased isokinetic strength and endurance in healthy adult women; however, neither zinc status nor dietary intake was evaluated before or after supplementation, weakening the study design (Krotkiewski et al. 1982).

In summary, it appears that zinc depletion impairs both maximal oxygen consumption and muscular endurance due to reductions in the activities of zinc-dependent enzymes. However, it is important to note that zinc intakes much less than the RDA (<4 mg/day) were used in the studies that demonstrated impaired performance (Van Loan et al. 1999; Lukaski 2005). And, as described below, such low intakes are rarely seen in survey studies of female athletes.

Dietary Zinc Intakes of Female Athletes

Similar to the difficulties described for measuring iron intakes of female athletes, assessment of dietary zinc intakes of female athletes is complicated by the evolution of recommended nutrient intakes over time and by differences in how investigators have defined adequacy. With the current DRIs, the EAR is the appropriate standard to evaluate nutrient intakes of groups (IOM 2000). Studies that were conducted prior to the establishment of the DRIs for zinc in 2000 (RDA 1989) often used two-thirds of the former RDA, which was 12 mg/day, as the criterion for sufficient dietary zinc intake. Based on this criterion, there is some evidence that many adolescent and collegiate female athletes do not consume adequate dietary zinc (Steen et al. 1995; Nuviala et al. 1999; Beals 2002; Papadopoulou et al. 2002; Ziegler et al. 2002; Hinton et al. 2004).

Zinc Deficiency

The progression of zinc deficiency is atypical of most micronutrients and varies significantly from that of iron. There is no storage form of zinc that can be mobilized in the face of reduced dietary intake or increased losses to preserve functional pools (Lukaski 2006). Consequently, loss of organ function occurs much earlier in zinc deficiency than is typically observed for other micronutrients. Rather than mobilization of stores, the homeostatic response to inadequate zinc intake is a reduction in both endogenous losses and growth. Zinc deficiency severe enough to manifest in clinical signs and symptoms in humans living in developed nations is rare. Zinc malabsorption due to the genetic disorder acrodermatitis enteropathica or to inflammatory bowel diseases can cause zinc deficiency. Severe malnutrition, such as occurs during anorexia nervosa, can also cause zinc deficiency. The consequences of zinc deficiency are diffuse and nonspecific: alopecia, diarrhea, eye and skin disorders, and loss of appetite (FNB 2001).

Athletes who follow a vegetarian or vegan diet are at increased risk for suboptimal intakes due to the lower absolute zinc content and bioavailability in plant foods (Craig and Mangels 2009). Vegetarians may need 1.5 times the RDA of zinc (FNB 2001). Likewise, athletes who restrict energy intake appear to be more likely to have suboptimal intakes of zinc (Lukaski 2004).

Assessment of Zinc Status

Due to the lack of a specific and sensitive indicator of whole-body zinc content, assessment of zinc status in humans is difficult. The concentration of zinc in plasma (or serum) is often used as an indicator, but this measure is insensitive to changes in dietary zinc or whole-body zinc status (Wada et al. 1985). Manore et al. (1993) found that plasma zinc did not respond to 12 weeks of zinc supplementation but was transiently altered with the initiation of regular exercise in a training-intensity-dependent manner. Similarly, the concentration of zinc in RBCs or mononuclear cells cannot be used to detect mild or moderate zinc deficiency (Lukaski 2004). Further complicating the assessment of zinc status in athletic populations is the fact that plasma

zinc concentration is acutely affected by exercise, food intake, inflammation, stress, life-cycle phase (e.g., adolescent growth spurt) and hormonal status (Cordova and Alvarez-Mon 1995; Fogelholm et al. 2000; Lukaski 2006). Serum metallothionein, a protein that binds zinc, responds to changes in dietary zinc (Grider et al. 1990). However, because metallothionein is made in the liver, serum concentrations are also affected by inflammation, stress, and exercise. Measurement of both serum zinc and serum metallothionein is a better indicator than metallothionein alone (King 1990). For example, low concentrations of both zinc and metallothionein suggest a reduction in zinc status, while a reduction in zinc, but not metallothionein, is indicative of zinc redistribution (not suboptimal status).

Prevention and Treatment of Zinc Deficiency

Consuming a Zinc-Adequate Diet

As discussed above, animal-based sources of dietary zinc (i.e., red meat, poultry, and dairy products) provide the majority of zinc in the typical American diet. Not only do those foods contain greater amounts of zinc per serving compared to plant-based foods, but the bioavailability of zinc is also greater. Thus, female athletes should be encouraged to consume these foods daily. Individuals who follow a vegetarian or vegan diet can obtain zinc from soy products, legumes, grains, and nuts (Craig and Mangels 2009). Zinc bioavailability can be enhanced by soaking beans prior to cooking or choosing leavened bread as opposed to unleavened bread (e.g., crackers, as leavening facilitates breakdown of phytates) (Craig and Mangels 2009). Zinc-deficient individuals may require treatment with supplemental zinc. Therapeutic adult doses typically range from 15 to 100 mg Zn/day.

Zinc Supplements

Supplemental zinc is available as zinc sulfate, gluconate, and acetate, and absorption does not appear to vary between the various zinc salts (Hambidge et al. 2010). Another potential source is zinc found in cold lozenges and intranasal sprays. Cold lozenges typically contain 10 to 25 mg zinc as zinc gluconate or acetate. In 2009, the U.S. Food and Drug Administration (FDA) advised consumers not to use zinc-containing sprays due to numerous reports of loss of sense of smell. There is not much information available on the use of zinc supplements among female athletes. However, two studies of female collegiate athletes reported that the prevalence of zinc supplementation was 3 to 4% (Froiland et al. 2004; Herbold et al. 2004).

Zinc Excess

The UL for zinc for adult women is 34 mg/day (FNB 2001). Ingestion of excess zinc (50 to 150 mg Zn/day) causes acute gastrointestinal problems: stomach pain, nausea, vomiting, diarrhea, loss of appetite, and abdominal cramps (FNB 2001). Excessive zinc intake reduces absorption of copper because these trace minerals have similar physiochemical properties. It is important to note that the interference does not require simultaneous ingestion of zinc and copper (Cordova and Alvarez-Mon 1995). Zinc supplementation at a daily dose of 22.5 mg impairs copper absorption, and 6

weeks of zinc supplementation at a dose of 50 mg/day induced copper deficiency (Fischer et al. 1984). High doses of zinc have also been shown to adversely affect blood lipids. One study reported that high doses of zinc reduce exercise-induced increases in HDL (Goodwin et al. 1985). Thus, female athletes should avoid supplemental zinc in amounts that exceed the RDA.

SUMMARY

Female athletes are at an increased risk for deficiencies of iron and zinc for a number of reasons, including inadequate intake, poor bioavailability, and increased losses. The high prevalence of clinical and subclinical iron deficiency among female athletes is concerning because of the significant impact this can have on both health and performance (and the fact that with education and proper nutritional intervention it could be fairly readily prevented and treated). From a practical perspective, dietary modifications that aim to improve iron status (i.e., increasing the intake of iron-rich meats, legumes, and fortified grains) will also increase dietary zinc intakes. Moreover, diet modification, rather than use of supplements, is the preferred strategy for ensuring adequate intake among female athletes because high doses of iron have the potential to be dangerously toxic and supplemental zinc may have detrimental effects on iron bioavailability. Thus, the use of supplemental iron or zinc to correct a deficiency should be undertaken only with supervision by a healthcare professional to monitor treatment efficacy and safety.

REFERENCES

Anderson, G. J., Darshan, D., Wilkins, S. J., and Frazer, D. M. 2007. Regulation of systemic iron homeostasis: How the body responds to changes in iron demand. *Biometals* 20(3-4): 665–674.

Beals, K. A. 2002. Eating behaviors, nutritional status, and menstrual function in elite female adolescent volleyball players. *J Am Diet Assoc*. 102(9): 1293–1296.

Beard, J., and Tobin, B. 2000. Iron status and exercise. *Am J Clin Nutr*. 72(2 Suppl): 594S–597S.

Beard, J. L. 2001. Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr*. 131(2S–2): 568S–579S; discussion 580S.

Beard, J. L., Hendricks, M. K., Perez, E. M., Murray-Kolb, L. E., Berg, A., Vernon-Feagans, L., Irlam, J., Isaacs, W., Sive, A., and Tomlinson, M. 2005. Maternal iron deficiency anemia affects postpartum emotions and cognition. *J Nutr*. 135(2): 267–272.

Brand, I. A., and Kleineke, J. 1996. Intracellular zinc movement and its effect on the carbohydrate metabolism of isolated rat hepatocytes. *J Biol Chem*. 271(4): 1941–1949.

Brownlie, T. T., Utermohlen, V., Hinton, P. S., and Haas, J. D. 2004. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. *Am J Clin Nutr*. 79(3): 437–443.

Brun, J. F., Dieu-Cambrezy, C., Charpiat, A., Fons, C., Fedou, C., Micallef, J. P., Fussellier, M., Bardet, L., and Orsetti, A. 1995. Serum zinc in highly trained adolescent gymnasts. *Biol Trace Elem Res*. 47(1–3): 273–278.

Brutsaert, T. D., Hernandez-Cordero, S., Rivera, J., Viola, T., Hughes, G., and Haas, J. D. 2003. Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women. *Am J Clin Nutr*. 77(2): 441–448.

Casimiro-Lopes, G., de Oliveira-Junior, A. V., Portella, E. S., Lisboa, P. C., Donangelo, C. M., de Moura, E. G., and Koury, J. C. 2009. Plasma leptin, plasma zinc, and plasma copper are associated in elite female and male judo athletes. *Biol Trace Elem Res*. 127(2): 109–115.

Celsing, F., Blomstrand, E., Werner, B., Pihlstedt, P., and Ekblom, B. 1986. Effects of iron deficiency on endurance and muscle enzyme activity in man. *Med Sci Sports Exerc*. 18(2): 156–161.

Chinevere, T. D., Kenefick, R. W., Cheuvront, S. N., Lukaski, H. C., and Sawka, M. N. 2008. Effect of heat acclimation on sweat minerals. *Med Sci Sports Exerc*. 40(5): 886–891.

Choe, Y. H., Kwon, Y. S., Jung, M. K., Kang, S. K., Hwang, T. S., and Hong, Y. C. 2001. *Helicobacter pylori*-associated iron-deficiency anemia in adolescent female athletes. *J Pediatr*. 139(1): 100–104.

Chung, C. S., Stookey, J., Dare, D., Welch, R., Nguyen, T. Q., Roehl, R., Peerson, J. M., King, J. C., and Brown, K. H. 2008. Current dietary zinc intake has a greater effect on fractional zinc absorption than does longer term zinc consumption in healthy adult men. *Am J Clin Nutr*. 87(5): 1224–1229.

Cook, J. D., and Finch, C. A. 1979. Assessing the iron status of a population. *Am J Clin Nutr.* 32(10): 2115–2119.

Cordova, A., and Alvarez-Mon, M. 1995. Behaviour of zinc in physical exercise: A special reference to immunity and fatigue. *Neurosci Biobehav Rev*. 19(3): 439–445.

Corwin, E. J., Murray-Kolb, L. E., and Beard, J. L. 2003. Low hemoglobin level is a risk factor for postpartum depression. *J Nutr*. 133(12): 4139–4142.

Cowell, B. S., Rosenbloom, C. A., Skinner, R., and Summers, S. H. 2003. Policies on screening female athletes for iron deficiency in NCAA division I-A institutions. *Int J Sport Nutr Exerc Metab*. 13(3): 277–285.

Craig, W. J., and Mangels, A. R. 2009. Position of the American Dietetic Association: Vegetarian diets. *J Am Diet Assoc*. 109(7): 1266–1282.

Davies, K. J., Maguire, J. J., Brooks, G. A., Dallman, P. R., and Packer, L. 1982. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. *Am J Physiol*. 242(6): E418–E427.

Earley, C. J., Allen, R. P., Beard, J. L., and Connor, J. R. 2000. Insight into the pathophysiology of restless legs syndrome. *J Neurosci Res*. 62(5): 623–628.

Eliakim, A., Nemet, D., and Constantini, N. 2002. Screening blood tests in members of the Israeli National Olympic team. *J Sports Med Phys Fitness*. 42(2): 250–255.

Fallon, K. E. 2004. Utility of hematological and iron-related screening in elite athletes. *Clin J Sport Med*. 14(3): 145–152.

Finch, C. A., Miller, L. R., Inamdar, A. R., Person, R., Seiler, K., and Mackler, B. 1976. Iron deficiency in the rat. Physiological and biochemical studies of muscle dysfunction. *J Clin Invest*. 58(2): 447–453.

Fischer, C. P. 2006. Interleukin-6 in acute exercise and training: What is the biological relevance? *Exerc Immunol Rev*. 12: 6–33.

Fischer, P. W., Giroux, A., and L'Abbe, M. R. 1984. Effect of zinc supplementation on copper status in adult man. *Am J Clin Nutr*. 40(4): 743–746.

FNB. 1989. *Recommended Dietary Allowances*. Washington, DC: National Academy of Sciences.

FNB. 2000. *Dietary Reference Intakes: Applications in Dietary Assessment,* Institute of Medicine. Washington, DC: National Academy of Sciences.

FNB. 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Nickel, Silicon, Vanadium, and Zinc,* National Academy of Sciences. Washington, DC: Institute of Medicine.

Fogelholm, M., Rankinen, T., Isokaanta, M., Kujala, U., and Uusitupa, M. 2000. Growth, dietary intake, and trace element status in pubescent athletes and schoolchildren. *Med Sci Sports Exerc*. 32(4): 738–746.

Froiland, K., Koszewski, W., Hingst, J., and Kopecky, L. 2004. Nutritional supplement use among college athletes and their sources of information. *Int J Sport Nutr Exerc Metab*. 14(1): 104–120.

Gardner, G. W., Edgerton, V. R., Senewiratne, B., Barnard, R. J., and Ohira, Y. 1977. Physical work capacity and metabolic stress in subjects with iron deficiency anemia. *Am J Clin Nutr*. 30(6): 910–917.

Goodwin, J. S., Hunt, W. C., Hooper, P., and Garry, P. J. 1985. Relationship between zinc intake, physical activity, and blood levels of high-density lipoprotein cholesterol in a healthy elderly population. *Metabolism* 34(6): 519–523.

Grider, A., Bailey, L. B., and Cousins, R. J. 1990. Erythrocyte metallothionein as an index of zinc status in humans. *Proc Natl Acad Sci USA*. 87(4): 1259–1262.

Gropper, S. S., Blessing, D., Dunham, K., and Barksdale, J. M. 2006. Iron status of female collegiate athletes involved in different sports. *Biol Trace Elem Res*. 109(1): 1–14.

Hallberg, L., Rossander-Hulten, L., Brune, M., and Gleerup, A. 1992. Calcium and iron absorption: Mechanism of action and nutritional importance. *Eur J Clin Nutr*. 46(5): 317–327.

Hambidge, K. M., Casey, C. E., and Krebs, N. F. 1986. Zinc. In Mertz, W. (Ed.), *Trace Elements in Human and Animal Nutrition,* vol. 2, 5th ed., pp. 1-137. New York: Academic Press.

Hambidge, K. M., Miller, L. V., Westcott, J. E., Sheng, X., and Krebs, N. F. 2010. Zinc bioavailability and homeostasis. *Am J Clin Nutr*. 91(5): 1478S–1483S.

Herbold, N. H., Visconti, B. K., Frates, S., and Bandini, L. 2004. Traditional and nontraditional supplement use by collegiate female varsity athletes. *Int J Sport Nutr Exerc Metab*. 14(5): 586–593.

Hinton, P. S., Giordano, C., Brownlie, T., and Haas, J. D. 2000. Iron supplementation improves endurance after training in iron-depleted, nonanemic women. *J Appl Physiol*. 88(3): 1103–1111.

Hinton, P. S., Sanford, T. C., Davidson, M. M., Yakushko, O. F., and Beck, N. C. 2004. Nutrient intakes and dietary behaviors of male and female collegiate athletes. *Int J Sport Nutr Exerc Metab*. 14(4): 389–405.

Hinton, P. S., and Sinclair, L. M. 2007. Iron supplementation maintains ventilatory threshold and improves energetic efficiency in iron-deficient nonanemic athletes. *Eur J Clin Nutr*. 61(1): 30–39.

Huang, S. H., Johnson, K., and Pipe, A. L. 2006. The use of dietary supplements and medications by Canadian athletes at the Atlanta and Sydney Olympic Games. *Clin J Sport Med*. 16(1): 27–33.

Hunt, J. R. 2003. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. *Am J Clin Nutr*. 78(3 Suppl): 633S–639S.

Institute of Medicine (IOM), Food and Nutrition Board. 2000. *Dietary Reference Intakes: Applications in Dietary Assessment.* Washington DC: National Academy of Sciences.

International Olympic Committee (IOC). 2009. The International Olympic Committee (IOC) consensus statement on periodic health evaluation of elite athletes: March 2009. *J Athl Train*. 44(5): 538–557.

Khaled, S., Brun, J. F., Micallel, J. P., Bardet, L., Cassanas, G., Monnier, J. F., and Orsetti, A. 1997. Serum zinc and blood rheology in sportsmen (football players). *Clin Hemorheol Microcirc*. 17(1): 47–58.

Kim, S. H., Kim, H. Y., Kim, W. K., and Park, O. J. 2002. Nutritional status, iron-deficiency-related indices, and immunity of female athletes. *Nutrition* 18(1): 86–90.

King, J. C. 1990. Assessment of zinc status. *J Nutr*. 120(Suppl. 11): 1474–1479.

Klingshirn, L. A., Pate, R. R., Bourque, S. P., Davis, J. M., and Sargent, R. G. 1992. Effect of iron supplementation on endurance capacity in iron-depleted female runners. *Med Sci Sports Exerc*. 24(7): 819–824.

Krotkiewski, M., Gudmundsson, M., Backstrom, P., and Mandroukas, K. 1982. Zinc and muscle strength and endurance. *Acta Physiol Scand*. 116(3): 309–311.

Leong, W. I., and Lonnerdal, B. 2004. Hepcidin, the recently identified peptide that appears to regulate iron absorption. *J Nutr*. 134(1): 1–4.

Looker, A. C., Dallman, P. R., Carroll, M. D., Gunter, E. W., and Johnson, C. L. 1997. Prevalence of iron deficiency in the United States. *JAMA* 277(12): 973–976.

Lukaski, H. C. 1989. Effects of exercise training on human copper and zinc nutriture. *Adv Exp Med Biol*. 258: 163–170.

Lukaski, H. C. 2004. Vitamin and mineral status: Effects on physical performance. *Nutrition* 20(7–8): 632–644.

Lukaski, H. C. 2005. Low dietary zinc decreases erythrocyte carbonic anhydrase activities and impairs cardiorespiratory function in men during exercise. *Am J Clin Nutr*. 81(5): 1045–1051.

Lukaski, H. C. 2006. Zinc. In Wolinsky, I., and Driskell, J. A. (Eds.), *Sports Nutrition: Vitamins and Trace Elements*, 2nd ed., pp. 217–234. Boca Raton, FL: CRC/Taylor & Francis.

Lukaski, H. C., Hoverson, B. S., Gallagher, S. K., and Bolonchuk, W. W. 1990. Physical training and copper, iron, and zinc status of swimmers. *Am J Clin Nutr*. 51(6): 1093–1099.

Manore, M. M., Helleksen, J. M., Merkel, J., and Skinner, J. S. 1993. Longitudinal changes in zinc status in untrained men: Effects of two different 12-week exercise training programs and zinc supplementation. *J Am Diet Assoc.* 93(10):1165–8.

Margeli, A., Skenderi, K., Tsironi, M., Hantzi, E., Matalas, A. L., Vrettou, C., Kanavakis, E., Chrousos, G., and Papassotiriou, I. 2005. Dramatic elevations of interleukin-6 and acute-phase reactants in athletes participating in the ultradistance foot race spartathlon: Severe systemic inflammation and lipid and lipoprotein changes in protracted exercise. *J Clin Endocrinol Metab*. 90(7): 3914–3918.

Martinović, J., Kotur-Stevuljević, J., Dopsaj, V., Dopsaj, M., Stefanović, A., and Kasum, G. 2010. Paraoxonase activity in athletes with depleted iron stores and iron-deficient erythropoiesis. *Clin Biochem*. 43(15): 1225–1229.

McCall, K. A., Huang, C., and Fierke, C. A. 2000. Function and mechanism of zinc metalloenzymes. *J Nutr.* 130(5S Suppl): 1437S–1446S.

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Nindl, B. C., Young, A. J., and Lieberman, H. R. 2009. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: Effects on iron status, physical performance, and mood. *Am J Clin Nutr*. 90(1): 124–131.

McInnis, M. D., Newhouse, I. J., von Duvillard, S. P., and Thayer, R. 1998. The effect of exercise intensity on hematuria in healthy male runners. *Eur J Appl Physiol Occup Physiol*. 79(1): 99–105.

McKie, A. T., Barrow, D., Latunde-Dada, G. O., Rolfs, A., Sager, G., Mudaly, E., Mudaly, M., Richardson, C., Barlow, D., Bomford, A., Peters, T. J., Raja, K. B., Shirali, S., Hediger, M. A., Farzaneh, F., and Simpson, R. J. 2001. An iron-regulated ferric reductase associated with the absorption of dietary iron. *Science* 291(5509): 1755–1759.

Mettler, S., and Zimmermann, M. B. 2010. Iron excess in recreational marathon runners. *Eur J Clin Nutr*. 64(5): 490–494.

Milic, R., Martinovic, J., Dopsaj, M., and Dopsaj, V. 2011. Haematological and iron-related parameters in male and female athletes according to different metabolic energy demands. *Eur J Appl Physiol*. 111(3): 449–458.

Nemeth, E., Valore, E. V., Territo, M., Schiller, G., Lichtenstein, A., and Ganz, T. 2003. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood* 101(7): 2461–2463.

Newhouse, I. J., Clement, D. B., Taunton, J. E., and McKenzie, D. C. 1989. The effects of prelatent/latent iron deficiency on physical work capacity. *Med Sci Sports Exerc*. 21(3): 263–268.

Nuviala, R. J., Lapieza, M. G., and Bernal, E. 1999. Magnesium, zinc, and copper status in women involved in different sports. *Int J Sport Nutr*. 9(3): 295–309.

Papadopoulou, S. K., Papadopoulou, S. D., and Gallos, G. K. 2002. Macro- and micro-nutrient intake of adolescent Greek female volleyball players. *Int J Sport Nutr Exerc Metab*. 12(1): 73–80.

Park, J. H., Grandjean, C. J., Antonson, D. L., and Vanderhoof, J. A. 1986. Effects of isolated zinc deficiency on the composition of skeletal muscle, liver and bone during growth in rats. *J Nutr*. 116(4): 610–617.

Peeling, P., Dawson, B., Goodman, C., Landers, G., and Trinder, D. 2008. Athletic-induced iron deficiency: New insights into the role of inflammation, cytokines and hormones. *Eur J Appl Physiol.* 103(4): 381–391.

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E. T., Swinkels, D. W., and Trinder, D. 2009a. Effects of exercise on hepcidin response and iron metabolism during recovery. *Int J Sport Nutr Exerc Metab*. 19(6): 583–597.

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E. T., Swinkels, D. W., and Trinder, D. 2009b. Training surface and intensity: Inflammation, hemolysis, and hepcidin expression. *Med Sci Sports Exerc*. 41(5): 1138–1145.

Perkkio, M. V., Jansson, L. T., Brooks, G. A., Refino, C. J., and Dallman, P. R. 1985. Work performance in iron deficiency of increasing severity. *J Appl Physiol*. 58(5): 1477–1480.

Pitsis, G. C., Fallon, K. E., Fallon, S. K., and Fazakerley, R. 2004. Response of soluble transferrin receptor and iron-related parameters to iron supplementation in elite, iron-depleted, nonanemic female athletes. *Clin J Sport Med*. 14(5): 300–304.

Punnonen, K., Irjala, K., and Rajamaki, A. 1997. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* 89(3): 1052–1057.

Recommended Dietary Allowances. 1989. Subcommittee on the 10th Edition of the Recommended Dietary Allowances, Food and Nutrition Board, Commission on Life Sciences, National Research Council. "Front Matter," *Recommended Dietary Allowances,* 10th edition. Washington, DC: The National Academies Press, 1989.

Rietjens, G. J., Kuipers, H., Hartgens, F., and Keizer, H. A. 2002. Red blood cell profile of elite Olympic distance triathletes. A three-year follow-up. *Int J Sports Med*. 23(6): 391–396.

Roecker, L., Meier-Buttermilch, R., Brechtel, L., Nemeth, E., and Ganz, T. 2005. Iron-regulatory protein hepcidin is increased in female athletes after a marathon. *Eur J Appl Physiol*. 95(5–6): 569–571.

Rowland, T. W., Deisroth, M. B., Green, G. M., and Kelleher, J. F. 1988. The effect of iron therapy on the exercise capacity of nonanemic iron-deficient adolescent runners. *Am J Dis Child*. 142(2): 165–169.

Rudzki, S. J., Hazard, H., and Collinson, D. 1995. Gastrointestinal blood loss in triathletes: Its etiology and relationship to sports anaemia. *Aust J Sci Med Sport.* 27(1): 3–8.

Schobersberger, W., Tschann, M., Hasibeder, W., Steidl, M., Herold, M., Nachbauer, W., and Koller, A. 1990. Consequences of 6 weeks of strength training on red cell O_2 transport and iron status. *Eur J Appl Physiol Occup Physiol*. 60(3): 163–168.

Schumacher, Y. O., Schmid, A., Grathwohl, D., Bultermann, D., and Berg, A. 2002a. Hematological indices and iron status in athletes of various sports and performances. *Med Sci Sports Exerc*. 34(5): 869–875.

Schumacher, Y. O., Schmid, A., Konig, D., and Berg, A. 2002b. Effects of exercise on soluble transferrin receptor and other variables of the iron status. *Br J Sports Med*. 36(3): 195–199.

Selby, G. B., and Eichner, E. R. 1986. Endurance swimming, intravascular hemolysis, anemia, and iron depletion. New perspective on athlete's anemia. *Am J Med*. 81(5): 791–794.

Sinclair, L. M., and Hinton, P. S. 2005. Prevalence of iron deficiency with and without anemia in recreationally active men and women. *J Am Diet Assoc*. 105(6): 975–978.

Skikne, B. S., Flowers, C. H., and Cook, J. D. 1990. Serum transferrin receptor: A quantitative measure of tissue iron deficiency. *Blood* 75(9): 1870–1876.

Steen, S. N., Mayer, K., Brownell, K. D., and Wadden, T. A. 1995. Dietary intake of female collegiate heavyweight rowers. *Int J Sport Nutr*. 5(3): 225–231.

Steensberg, A., Febbraio, M. A., Osada, T., Schjerling, P., van Hall, G., Saltin, B., and Pedersen, B. K. 2001. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol.* 537(Pt 2): 633–639.

Stupnicki, R., Malczewska, J., Milde, K., and Hackney, A. C. 2003. Day to day variability in the transferrin receptor/ferritin index in female athletes. *Br J Sports Med*. 37(3): 267–269.

Suominen, P., Punnonen, K., Rajamäki, A., and Irjala, K. 1998. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. *Blood* 15;92(8): 2934–2939.

Tanaka, T., Roy, C. N., Yao, W., Matteini, A., Semba, R. D., Arking, D., Walston, J. D., Fried, L. P., Singleton, A., Guralnik, J., Abecasis, G. R., Bandinelli, S., Longo, D. L., and Ferrucci, L. 2010. A genome-wide association analysis of serum iron concentrations. *Blood* 115(1): 94–96.

Telford, R. D., Sly, G. J., Hahn, A. G., Cunningham, R. B., Bryant, C., and Smith, J. A. 2003. Footstrike is the major cause of hemolysis during running. *J Appl Physiol*. 94(1): 38–42.

Toft, A. D., Falahati, A., and Steensberg, A. 2010. Source and kinetics of interleukin-6 in humans during exercise demonstrated by a minimally invasive model. *Eur J Appl Physiol*. 111(7): 1351–1359.

Vallee, B. L., and Falchuk, K. H. 1993. The biochemical basis of zinc physiology. *Physiol Rev*. 73(1): 79–118.

Van Loan, M. D., Sutherland, B., Lowe, N. M., Turnlund, J. R., and King, J. C. 1999. The effects of zinc depletion on peak force and total work of knee and shoulder extensor and flexor muscles. *Int J Sport Nutr*. 9(2): 125–135.

Wada, L., Turnlund, J. R., and King, J. C. 1985. Zinc utilization in young men fed adequate and low zinc intakes. *J Nutr*. 115(10): 1345–1354.

Weight, L. M., Byrne, M. J., and Jacobs, P. 1991. Haemolytic effects of exercise. *Clin Sci (Lond)*. 81(2): 147–152.

Weight, L. M., Darge, B. L., and Jacobs, P. 1991. Athletes' pseudoanaemia. *Eur J Appl Physiol Occup Physiol*. 62(5): 358–362.

Wilkinson, J. G., Martin, D. T., Adams, A. A., and Liebman, M. 2002. Iron status in cyclists during high-intensity interval training and recovery. *Int J Sports Med*. 23(8): 544–548.

Willis, W. T., Brooks, G. A., Henderson, S. A., and Dallman, P. R. 1987. Effects of iron deficiency and training on mitochondrial enzymes in skeletal muscle. *J Appl Physiol*. 62(6): 2442–2446.

Woodson, R. D., Wills, R. E., and Lenfant, C. 1978. Effect of acute and established anemia on O_2 transport at rest, submaximal and maximal work. *J Appl Physiol*. 44(1): 36–43.

Woolf, K., St Thomas, M. M., Hahn, N., Vaughan, L. A., Carlson, A. G., and Hinton, P. 2009. Iron status in highly active and sedentary young women. *Int J Sport Nutr Exerc Metab*. 19(5): 519–535.

Zhu, Y. I., and Haas, J. D. 1998a. Altered metabolic response of iron-depleted nonanemic women during a 15-km time trial. *J Appl Physiol*. 84(5): 1768–1775.

Zhu, Y. I., and Haas, J. D. 1998b. Response of serum transferrin receptor to iron supplementation in iron-depleted, nonanemic women. *Am J Clin Nutr*. 67(2): 271–275.

Ziegler, P., Sharp, R., Hughes, V., Evans, W., and Khoo, C. S. 2002. Nutritional status of teenage female competitive figure skaters. *J Am Diet Assoc*. 102(3): 374–379.

6 Nutrients Needed for Optimal Bone Health in the Female Athlete

Kristine Spence

CONTENTS

INTRODUCTION

Far from inert and static, the skeleton is constantly in motion, and this perpetual remodeling is orchestrated by the coordination of multiple body systems. From neural stimulation to sympathetic response and cellular action, the cascade of reactions that enables bone remodeling is tightly regulated by energy metabolism and the availability of essential nutrients. Beginning with embryonic development and progressing throughout the life cycle, nutrition, at both the macronutrient and micronutrient levels, plays a key role in the health and integrity of bone structure. Exactly

how bone maintains its integrity and the intervariability between people is a complex science, dependent upon myriad genetic, lifestyle, and dietary components, that is not yet fully understood. However, recent, cross-disciplinary research from both applied and basic scientists has begun to piece together the puzzle.

Understanding how nutrition impacts bone is essential to the female athlete for several reasons. At its most rudimentary level, skeletal turnover is governed by two primary factors: serum calcium levels determine *when* remodeling takes place and skeletal stress determines *where* remodeling takes place. Dietary quality impacts the availability of calcium within the body, and exercise type, duration, and intensity determine skeletal stress. As the female athlete engages in sport, she subjects her body to various skeletal stressors, yet without adequate nutrition, bone cannot respond properly and remodeling uncouples. This may lead to increased risk of injury, failure to achieve peak bone mass, or in severe cases, osteoporotic fracture.

For the young, female athlete, attention to nutrition during the adolescent years is of utmost importance. The onset of puberty is a critical time for bone development, as the introduction of sex hormones dramatically increases growth and bone accretion. Young females achieve ~37% of peak bone mass between the ages of 11 and 15 years (Matkovic et al. 1994). By the age of 20, young adults have achieved 90% of peak bone mass (PBM), a fact that underscores the importance of optimizing bone health at this critical age (Heaney 2009).

This chapter will explore the various micro- and macronutrients that impact bone health and will offer insight into the most recent research and recommendations for the female athlete. Before discussing the impact of individual nutrients; however, it is necessary to paint the big picture. The relationship between nutrition and bone health is ultimately dependent on total energy intake. Energy metabolism and bone metabolism are inexorably linked, so the *amount* of food an athlete consumes is of fundamental importance.

ENERGY INTAKE AND AVAILABILITY

The body requires a certain amount of energy from dietary calories in order to function. Each of the body's operational tasks from basic, involuntary metabolism (i.e., pulmonary function, cellular respiration, cardiac function, etc.) to voluntary locomotion, including exercise, has an energy cost. As female athletes increase exercise duration and intensity, they up the ante and require additional calories to support increases in energy expenditure. The concept of energy availability (discussed in detail in Chapters 8–11, which address the Female Athlete Triad) is defined as dietary energy intake minus exercise energy expenditure (Ihle and Loucks 2004). When energy availability is low, the body does not have enough calories to support the energy demands of metabolism plus the added demand of exercise. The result is a potentially damaging cascade of hormonal, neurological, and physiological reactions that can lead to compromised bone health (Gordon 2010).

For many years, prevailing wisdom suggested that hypoestrogenism was the primary cause of bone loss in amenorrheic young women; however, recent research on female athletes with low energy availability suggests that energy metabolism and the availability of adequate calories are at the root of maintaining bone health through

its impact on both hormonal and metabolic pathways. In an observational study of 44 exercising premenopausal women, De Souza et al. showed that as long as energy intake was adequate, estrogen status did not impact bone turnover (De Souza et al. 2008). Inadequate energy intake was, however, associated with bone loss due to decreased formation as well as increased resorption (De Souza et al. 2008). This research suggests that available energy may be the key modulator to sustaining bone health.

Because bone remodeling demands a significant amount of energy, the skeleton requires an *energy balance sensor* to determine whether or not enough energy is available for remodeling to occur. New research is beginning to explore the connection between bone health and energy availability including leptin-dependent physiological mechanisms that regulate both weight and bone (Karsenty 2006; Rosen 2008; Confavreux 2011). Evidence of a potential leptin-modulated "sensor" comes from piecing together long-standing clinical observations with recent scientific advancements in genetics (Karsenty 2006; Confavreux 2011). Peripheral adipocytes release leptin, which sparks neural pathways linked to bone building (Karsenty 2006). Female athletes who suffer from hypothalamic amenorrhea exhibit decreased leptin, which may result in hypoestrogenism (De Souza et al. 2008; Gordon 2010). Exogenous leptin administration in energy-deficient females results in restored ovulation and improved markers of bone formation. Nutritional therapy accomplishes the same result, providing further evidence of the direct connection shared between energy intake, energy availability, and bone remodeling (De Souza et al. 2008).

These emerging connections bring to the forefront the importance of adequate energy intake for optimizing bone health and offer a strong platform for those working directly with young female athletes. As described by Ihle and Loucks (2004) and cited in the American College of Sports Medicine's 2007 Position Stand on the female athlete triad (Nattiv et al. 2007), dipping below the threshold of 30 kcal/kgLBM/day seems to be the point at which reproductive and bone health begin to suffer. Athletes looking to maintain weight and achieve optimal bone health should target 45 kcal/kgLBM/day (Manore et al. 2007).

BONE NUTRIENTS

Having established this foundation and highlighted the direct relationship between energy availability and bone health, we now focus on the source of these calories for optimizing skeletal health. For practitioners and clinicians who work directly with female athletes, it is essential to keep in mind that applying this information to athletes' dietary *patterns* is crucial. While there are various nutrients that have been isolated as playing a particular role in enhancing bone health, many of these nutrients interact synergistically within whole foods. So, when making recommendations and discussing nutritional strategies to improve bone health in female athletes, health professionals should remember to talk about *foods* and *dietary patterns* instead of honing in too closely on individual nutrients. Also, it should be emphasized that in many female athletes, especially those suffering from low energy availability, inadequate energy intake means a deficit in macro- and micronutrients, so advocating a nutrient-dense diet is imperative. Based on our current understanding of bone metabolism, the following sections describe the impact specific macro- and micronutrients have on skeletal integrity.

Dietary Components Believed to Positively Impact Bone Health

Protein

With half of the material in the bone matrix derived from protein, it is not surprising that a steady supply of dietary protein is essential for maintaining structural integrity during the remodeling process. The ideal amount of protein for optimizing bone health and reducing long-term fracture risk has been an actively debated topic in the literature (Bonjour 2005; Darling et al. 2009; Cao and Nielsen 2010), with one camp suggesting that excessive protein intake induces metabolic acidosis and a subsequent increase in bone resorption, while others maintain that higher protein boosts markers for bone health. The 2010 Dietary Guidelines for Americans maintain an Acceptable Macronutrient Distribution Range (AMDR) for protein between 10 and 35% of total calories, providing for widely different dietary patterns. The breadth of this range raises questions about where to direct female athletes.

For optimal bone health, it appears that a higher protein intake earlier in life (i.e., <30 years) has a greater impact on bone mineral density (BMD) than in women who have already achieved PBM (i.e., 30 to 40 years) (Beasley et al. 2010); however, in older women (women over 50, especially postmenopausal), protein needs again increase in order to maintain bone health (Bonjour 2005). Calcium status may also impact how protein affects bone. In postmenopausal women with suboptimal calcium intakes (~675 mg/day), dietary protein boosted intestinal absorption, an effect not observed with calcium adequacy (~1510 mg/day) (Hunt et al. 2009).

That higher protein diets result in increased urinary calcium excretion has long been held as evidence supporting the detrimental effects of a high protein intake on bone health. The theory behind this hypothesis, sometimes referred to as the *acid-ash* hypothesis, suggests that an increase in acid production from a high-protein diet (especially animal proteins, which are rich in the sulfur-containing amino acids cysteine and methionine) creates a metabolically acidic environment to which the skeleton responds by boosting bone resorption and releasing calcium as a buffer (Cao and Nielsen 2010). Nonetheless, in recent years, this theory has been challenged. Examining only calciuria as a marker of protein's impact on bone health fails to take into consideration the complete pathophysiological impact of protein consumption. Dietary protein (both from animal and vegetable sources) increases urinary calcium absorption, stimulates circulating levels of IGF-1 (a hormone responsible for boosting the activity of bone-building osteoblasts), and reduces serum parathyroid hormone (PTH), which results in decreased bone resorption (Bonjour 2005). Each of these parameters demonstrates how protein intake positively impacts bone formation.

To further elucidate this relationship between protein intake and its effect on calcium balance, Kerstetter et al. conducted an intervention with 13 women (10 premenopausal, 3 postmenopausal) and showed that when calcium intake remains constant, adjusting dietary protein from 1 g/kg/day to 2.1 g/kg/day results in an 8% increase in calcium absorption. This increase in absorption directly offsets the demonstrated rise in calcium excretion, yielding no change in net calcium balance at these two different protein levels (Kerstetter et al. 2005).

Epidemiological data suggest that a higher protein intake correlates with improved BMD in the long term (Heaney 2009), and two recent meta-analyses help highlight

these observations. Darling et al. examined 61 studies of diverse design and population groups demonstrating a positive association between protein intake, bone strength, and bone density, and an inverse relationship between protein intake and bone resorption (Darling et al. 2009). A second, rigorous meta-analysis by Fenton et al. showed that in adults, measures of urinary calcium excretion do not accurately reflect changes in overall calcium balance, concluding that promotion of the acid-ash hypothesis is not scientifically supported (Fenton et al. 2009).

Questions about the effects of animal versus vegetable protein on bone health are addressed in Bonjour's (2005) comprehensive review, where she suggests that there are no deleterious effects of animal-derived proteins on bone health. Reviewing the chemical analysis of various animal and vegetable proteins does not denote consistent differences in sulfur content. In fact, milk protein contained only half the sulfur content found in most cereal proteins (Bonjour 2005). This is of particular importance to female athletes who may choose vegetarian-type diets on the perceived basis that animal proteins will negatively impact bone health. In a 7-week randomized crossover trial, Roughead et al. tested the effects of substituting 25 mg of soy protein for an equivalent amount of meat protein on calcium retention. At the end of the intervention there was no difference in calcium retention or indicators of bone turnover (Roughead et al. 2005).

Due to sport-specific demands and increased energy expenditure, female athletes have higher protein needs than their sedentary counterparts. Couple this with growth demands in the adolescent athlete and protein needs increase further. Health and nutritional professionals working with the female athlete population should consider adequate protein as part of the athlete's overall dietary plan that includes a balance of all nutrients essential for bone health. Care should be taken with the vegetarian athlete to rule out any potential red flags associated with this dietary pattern (i.e., poor energy availability, menstrual dysfunction, disordered eating, pressure to maintain a certain body type). The nutrition professional should work with this athlete to determine adequate overall protein intake and diet quality and assess the athlete's motivation for her dietary choices. Despite some epidemiological data, namely the EPIC study that found a positive association between vegetable protein and bone health (Weikert et al. 2005), it does not seem that animal proteins negatively impact bone health or that a diet higher in vegetable protein is better for bone health (Bonjour 2005; Roughead et al. 2005; Cao and Nielsen 2010).

Fat

The release of leptin from the adipocyte may play a key role in signaling neural pathways that control hormonal regulation and impact bone metabolism. While in transit to the brain, leptin interacts with serotonin before binding to receptors in the hypothalamus (Karsenty 2006; Rosen 2008; Confavreux 2011). From here two neural pathways are activated that impact osteoblast cell differentiation (Karsenty 2006). Female athletes who suffer from hypothalamic amenorrhea exhibit decreased leptin (Gordon 2010). Furthermore, in female athletes, low energy availability is often associated with decreased fat mass. Because bone health is dependent upon adipokines (i.e., leptin) and fat-regulated hormones like ghrelin, it remains unclear what level of fat mass is necessary for normal metabolic, reproductive, and bone

function, and it is likely that it may be highly individualized (Russell and Misra 2010). *In vivo* studies on dietary fat and its impact on bone health are limited.

We do know that when bone turnover is uncoupled and resorption exceeds formation, the body releases a cascade of pro-inflammatory cytokines (Lacativa and Farias 2010). Classified thus as an inflammatory disease, treating osteoporosis with anti-inflammatory nutrition therapy seems logical. Consumption of polyunsaturated fatty acids, like omega-3 fatty acids, may reduce urinary calcium excretion, boost calcium absorption, and stimulate osteocalcin activity (Salari et al. 2008); however, results and human feeding studies remain too sparse to make definitive recommendations. Omega-3-rich foods, including fatty fish, walnuts, and flax seeds, are warranted for their overall nutrient profile that includes calcium, magnesium, protein, and vitamin D, if not for the anti-inflammatory fatty acids alone.

Calcium

As the body's most abundant mineral, calcium's physiological role in bone health is primarily structural as it provides the crystalline rigidity necessary for proper skeletal and dental mechanics. But calcium also exerts critical metabolic functions that impact protein utilization, nerve transmission, muscle contraction, blood clotting, and cellular communication, which is why serum calcium levels are so tightly regulated (Heaney 2009). Perhaps even more important than its structural role in the body, bone serves as a reservoir for calcium, a storage pool to draw from when calcium intake is inadequate to meet needs. The regulation of serum calcium is hormonally controlled by PTH, calcitonin, and vitamin D.

Responding to a drop in serum calcium (Ca^{2+}), PTH is secreted from the parathyroid gland to stimulate osteoclast activity and bone resorption, which, among other things, results in a release of calcium into the serum. PTH also acts to increase phosphate excretion and calcium reabsorption by the kidney in addition to activating vitamin D to stimulate an increase in intestinal calcium absorption (Heaney 2009). An increase in serum calcium stimulates the secretion of calcitonin from the thyroid gland, which acts directly on osteoclasts to inhibit bone resorption and stimulate calcium deposition in the matrix (Marieb 2004; Heaney 2009). An increase in dietary calcium stimulates the latter of these two events.

During the young adult and adolescent years when bone is growing most rapidly, especially in the 2 to 3 years surrounding puberty, calcium balance reaches its positive peak at 200 to 400 mg/day (Greydanus et al. 2010; Ross and U.S. Institute of Medicine 2011). This is a sign that formation is outpacing resorption and the reason why dietary guidelines are highest in this age group at 1300 mg/day. Data consistently show that adequate calcium consumption during these peak growing years contributes to attainment of PBM, reduces age-related bone loss, and decreases risk for fragility fractures both in youth and adults (Ilich and Kerstetter 2000; Heaney 2007). In fact, retrospective studies demonstrate that childhood calcium intake is a predictor of adult bone mass (Ilich and Kerstetter 2000). Results from NHANES data (2005–2006) show that calcium intake among females is consistently lower than Dietary Guidelines recommendations and lower than those of their male counterparts. Specifically, 90% of females ages 14 to 18 years failed to meet the 1997 Adequate Intake (AI) level of 1300 mg/day. In younger girls ages 9 to 13 years, 88%

fail to meet the AI; in older women, ages 19 to 30 years, 72% fall short of recommendations (Moshfegh et al. 2009). In a group of 39 high school runners, Barrack et al. identified that 50% of those with normal bone turnover failed to achieve a daily intake of 1300 mg Ca/day. In runners with heightened bone turnover, 85% had less than adequate intakes of dietary calcium (Barrack et al. 2010). Health professionals should be aware of these staggering inadequacies and remain vigilant of athletes' dietary patterns that may further decrease calcium intake (i.e., vegetarianism or veganism or other patterns in which dairy consumption is low).

Osteoporosis is more than a disease characterized by low bone mass—it is closely associated with accelerated remodeling and rapid resorption. Greater dietary calcium suppresses PTH secretion and reduces the speed of remodeling (Heaney 2009). However, there is a threshold at which additional dietary calcium no longer positively impacts bone health and it is estimated to be 1500 mg/day in adolescents and 1100 mg/day in adults (Ilich and Kerstetter 2000). This threshold must be taken into account when assessing athletes and evaluating research. Understanding an athlete's current intake level is imperative for making food and supplement recommendations. A second threshold exists for calcium absorption where serum vitamin D 25(OH)D is the limiting factor (Heaney 2007). The optimal level of vitamin D for maximal calcium absorption and overall skeletal health continues to be an actively debated topic that will be addressed in greater detail later in this chapter (Aloia et al. 2010; Heaney and Holick 2011).

Calcium is a unique nutrient in that the upper limit of its skeletal reserve is not dependent upon net calcium intake but on bone's mechanical load (Heaney 2001). This helps explain how exercise exerts an independent and positive effect on skeletal health. In female athletes, adequate calcium intake may help optimize the positive effects of exercise on bone (Hind and Burrows 2007), yet research consistently shows that young females fail to meet calcium recommendations (Heaney 2007; Moshfegh et al. 2009; Barrack et al. 2010). Because estrogen facilitates calcium movement into the bone, adolescent females with delayed menarche tend to have lower BMD than their normal menstruating counterparts and may be at increased risk for fragility stress fractures and early-onset osteoporosis if corrective action is not taken (Greydanus et al. 2010). Not only should adequate dietary calcium be stressed in this population, supplementation may be necessary in cases where diet is inadequate.

Working with this population can be challenging. Many young female athletes, especially those involved in lean-build and aesthetic sports, may be resistant and fearful of increasing calories. Thus, the sport dietitian and physician are encouraged to stress a diet with optimal nutrient density to help these athletes meet their needs. Please refer to Table 6.1 for foods rich in bone-building nutrients.

Vitamin D

There are very few foods naturally rich in vitamin D, and thus most is synthesized in the skin as a result of exposure to UVB sun rays. Societal shifts in work environment and greater attention to and awareness of the sun's negative impact on skin health have contributed toward less sun exposure and spawned a surge of research in the last decade on the possible implications of vitamin D deficiency and its effects on our current population (Holick 2007). While this research has identified a host of

TABLE 6.1
Bone-Specific Nutrients and DRI Recommendations/Nutrient Composition of Foods to Promote Bone Health

Recommendations (Females)	Protein g/kg/d	Calcium mg/d (RDA)	Fluoride mg/d (AI)	Iron mg/d (RDA)	Magnesium mg/d (RDA)	Phosphorus mg/d (RDA)	Potassium mg/d (AI)	Sodium mg/d (AI)	Zinc mg/d (RDA)	Vit. D[1] µg/d (RDA)	Vit. K µg/d (AI)	Vit. C mg/d (RDA)	Vit. A[2] (RAE) µg/d (RDA)
9–13 y	0.76	1300	2	8	240	1250	4500	1500	8	15	60	45	600
14–18 y	0.71	1300	3	15	360	1250	4700	1500	9	15	75	65	700
19–30 y	0.66	1000	3	18	310	700	4700	1500	8	15	90	75	700
31–50 y	0.66	1000	3	18	320	700	4700	1500	8	15	90	75	700
51–70 y	0.66	1200	3	8	320	700	4700	1300	8	15	90	75	700
>70 y	0.66	1200	3	8	320	700	4700	1200	8	20	90	75	700

Foods (Amount)	Protein g	Calcium mg	Fluoride Mg*	Iron mg	Magnesium mg	Phosphorus mg	Potassium mg	Sodium mg	Zinc mg	Vit. D µg	Vit. K µg	Vit. C mg	Vit. A (RAE) µg
Almonds (1 oz)	6	75	—	1	76	137	200	—	.87	—	—	—	—
Almond beverage (1 c)	1	450	—	.36	12.4	14	180	150	.16	3.75	—	—	70
Apricots (100 g ~1/3 c)	1.2	19	—	.94	11	25	411	4	.14	—	1.1	0.3	64
Bell pepper, red (1/2 c)	0.75	5	—	.32	9	19	157	3	.19	—	3.7	95	117
Black beans (1/2 c)	7	42		2.3	42	130	369	400	.65	—	—	3.3	—
Broccoli (1 c raw)	2.6	43	—	.66	19	60	288	30	.37	—	92.5	81.2	28
Cereal, fortified (1 c) (i.e., Cheerios)	3.2	114	—	8.9	40	122	171	160	4.4	1	0.9	6.8	242
Cheese, mozzarella, part skim (1 oz)	7	222	—	.06	7	131	24	175	.78	0.1	0.5	0	36

Chicken, breast (100 g ~3 oz)	31	15	—	1.04	29	228	256	74	1	-.1	0.3	0	6
Kale (1 c)	2.21	90	—	1.14	23	38	299	29	.29	0	547.4	80.4	515
Milk, skim (1 c)	8.26	299	—	0.07	27	247	382	103	1.03	2.9	0	0	149
Milk, chocolate lowfat (1 c)	8.1	290	—	0.68	32	258	425	152	1.02	2.8	0.2	2.2	145
Mushrooms (1/2 c)	1.08	1	—	0.17	3	30	111	2	0.18	0.1	0	0.7	0
Oatmeal (3/4 c, cooked)	4.45	16	—	1.57	47	135	122	7	1.75	0	0.5	0	0
Orange juice, fortified (1 c)	1.69	500	—	0.32	27	117	443	5	0.17	3.5	0	83.7	5
Pasta, whole wheat (1 c)	7.46	21	—	1.48	42	125	62	4	1.13	0	1.0	0	0
Peanut Butter (2 Tbsp)	7.7	14	—	0.61	51	102	238	5	0.89	0	0.2	0	0
Potato (1 med)	4.5	31	—	1.85	52	123	952	24	0.61	0	3.5	14.4	2
Romaine lettuce (1 c)	0.58	16	—	0.46	7	14	116	4	0.11	0	48.2	1.9	205
Salmon, wild (3 oz)	21.62	13	—	0.88	31	218	534	48	0.70	^	—	0	11
Soy beverage, all flavors (1 c)	7	199	—	1.07	—	151	156	90	—	—	2.4	0	151
Steak, lean (3 oz)	19.98	9	—	2.19	20	182	322	78	4.68	—	—	0	0
Tofu (1/2 cup)	19.88	861	—	3.35	73	239	299	18	1.98	0	—	0.3	—
Tuna, canned (3 oz)	20.08	12	—	0.82	28	184	201	320	0.41	1.7	2.1	0	5
Yogurt, plain (1 c)	14.04	488	—	0.22	47	385	625	189	2.38	0	0.5	2.2	5
Walnuts (1 oz)	4.32	28	—	0.82	45	98	125	1	0.88	0	0.8	0.4	0

Sources: DRI Reference Tables Food and Nutrition Board, Institute of Medicine, National Academies. U.S. Department of Agriculture, Agricultural Research Service, USDA Nutrient Data Laboratory, 2011, USDA National Nutrient Database for Standard Reference, Release 24, USDA Nutrient Data Laboratory Web site: http://www.ars.usda.gov/nutrientdata.

[1] Vit. D recommendations are for cholecalciferol (Vitamin D3); 1 µg = 40 IU.

[2] Vit. A recommendations are for retinol activity equivalents (RAEs); 1 RAE = 1 µg retinol, 12 µg β-carotene, 24 µg α-carotene, 24 µg β-cryptoxanthin.

[*] Fluoride values dependent upon water source.

[^] The value for Vit. D is not provided for whole fish or fillets, since it can vary greatly. 100 g of canned salmon (drained solids, minus bones and skin) contains 21.5 µg.

potential metabolic areas in which vitamin D appears to act as a modulator, those areas continue to be elucidated and are beyond the scope of this chapter. Instead, this section will focus on the relationship between vitamin D and bone health and highlight why vitamin D may be of particular importance to the female athlete.

That vitamin D plays an essential role in skeletal health is unequivocal; however, optimal levels of serum vitamin D for calcium absorption and bone remodeling continue to be debated. A committee of 14 scientists assembled by the Institute of Medicine (IOM) recently released its updated report on calcium and vitamin D and, based on the available science, has shifted the intake recommendation from an AI value to a Recommended Dietary Allowance (RDA) and boosted the appropriate level of daily vitamin D from 200 to 600 IU for most Americans (Ross and U.S. Institute of Medicine 2011). At this daily value, most people will maintain a serum 25(OH)D concentration of 20 ng/ml, which according to the IOM committee, is the level sufficient to ensure bone health. Nonetheless, a number of key researchers in this field disagree and believe that the minimum level for optimal bone and overall health should be set at a minimum of 30 ng/ml (Heaney and Holick 2011), and some propose higher (Cannell et al. 2006, 2009; Halliday et al. 2011).

Research shows that vitamin D is essential for calcium and phosphorus absorption. Without it, only 10 to 15% of dietary calcium and 60% of dietary phosphorus is absorbed (Holick 2007). When vitamin D levels are maintained above 30 ng/ml, fractional calcium absorption is twice what it is in a vitamin D–deficient state (Larson-Meyer and Willis 2010). Furthermore, vitamin D plays a key role in the endocrine modulation of calcium and phosphorus metabolism (Ilich and Kerstetter 2000). At serum levels below 10 ng/ml, PTH increases, which as described previously, correlates negatively with bone density (Larson-Meyer and Willis 2010). Vitamin D also improves neuromuscular function and reduces risk for falls in the elderly (Heaney 2007; Holick 2007). Among active individuals, vitamin D is important for the prevention of bone injury as identified by a recent study of male, Finnish military recruits where fracture risk increased when serum vitamin D dropped below 30 ng/ml (Ruohola et al. 2006). A blinded intervention trial in female naval recruits showed reduced stress fracture incidence following supplementation with 800 IU vitamin D and 2000 mg calcium (Lappe et al. 2008). Each of these points demonstrate vitamin D's role in bone metabolism; however, it is only just recently that scientists have begun exploring the literature and conducting novel research that focuses on athlete-specific needs. It seems that the impact of vitamin D extends well beyond its role in bone health.

Research from Germany and Russia between the 1920s and 1950s describes improved athletic performance among subjects receiving ultraviolet (UV) radiation via sunlamp, especially during the winter months (Cannell et al. 2009). These findings have seasonal and environmental applications for the competitive athlete, and Halliday et al. (2011) identified distinct seasonal variations in serum 25(OH)D concentrations among a group of college athletes (fall: 49 ± 16.6 ng/ml; winter: 30.5 ± 9.4 ng/ml; spring: 41.9 ± 14.6 ng/ml). If performance is altered by vitamin D status, attention to adequate vitamin D during sun-starved winter months, especially at northern latitudes, may be warranted, and due to the limited amount of vitamin D in foods, this may require supplementation. Furthermore, for those athletes competing

and training primarily indoors, their opportunity for sun exposure and therefore vitamin D synthesis may be limited. Couple this seasonal variability with additional findings that link vitamin D to immunity, inflammation, and muscle function (Willis et al. 2008), and we begin to see how compromised vitamin D status may impact the athlete's overall health and training consistency (Willis et al. 2008).

Though more research needs to be done in her study on college athletes, Halliday et al. (2011) identified a significant correlation between low vitamin D status and frequency of illness. Because illness compromises training integrity and consistency, potential dietary efforts to reduce incidence should be explored. Much of the research that exists linking skeletal pain and vitamin D has been done in the elderly; however, reports show consistent correlation between low serum 25(OH)D and nonspecific musculoskeletal pain and weakness (Larson-Meyer and Willis 2010). Trials in injured athletes have yet to be conducted to determine what, if any, role vitamin D status plays in this population.

We do know that many athletes are vitamin D insufficient. A study of dancers and athletes in Israel identified that 73% of the study population were vitamin D insufficient (<30 ng/ml), with higher prevalence among athletes participating in winter and indoor sports (Constantini et al. 2010). The review on vitamin D and the athlete by Larson-Meyer and Willis (2010) identified a wide range (4 to 32 ng/ml) of serum concentrations depending on when (time of year) the measures were taken and where (latitude) the athletes lived. The ideal serum concentration of 25(OH)D for optimal performance among a *female* athlete population remains unclear; however, the consensus among the existing literature is for ranges between 30 and 50 ng/ml (Cannell et al. 2009; Larson-Meyer and Willis 2010; Halliday et al. 2011; Heaney and Holick 2011).

Assessing athletes' vitamin D status among other biochemical factors is suggested for determining an overall, multidisciplinary approach to optimizing performance and health. Practitioners should also pay attention to the environmental conditions in which athletes train and compete (i.e., latitude, indoor, outdoor, time of day, etc.) and inquire about sunscreen use; this information will help assess status and guide recommendations. The latest IOM recommendations do not offer specific sun exposure guidelines (Ross and U.S. Institute of Medicine 2011); however, sensible sun exposure has been described as exposure of the arms and legs for 5 to 30 minutes twice per week, with duration dependent upon latitude, time of day, and season (Holick 2007). Recommendations for dietary intake should include those foods rich in vitamin D, namely fatty fish and fortified dairy products. (See Table 6.1 for a more comprehensive list.) Particular attention should again be given to athletes with specific dietary restrictions or disordered eating habits. In many cases, including winter months, supplementation may be the best course of action to maintain athletic performance and health throughout the year. Daily intakes of 800 to 2000 IU per day are common (Willis et al. 2008) and still well below the IOM committee's established upper limit (UL) of 4000 IU per day (Ross and U.S. Institute of Medicine 2011).

Phosphorus

Next to calcium, phosphorus is the second-most abundant mineral in the body, 85% of which is found bound to the crystal structure of the bone matrix (Ilich and Kerstetter

2000). While phosphorus is undoubtedly essential to the proper formation of bone structure, when high intake is accompanied by a low calcium intake, PTH activity increases and may potentially contribute to decreased BMD and subsequent fracture risk (Heaney 2009). Western dietary habits exhibit phosphorus intakes that often far exceed the recommended level of 700 mg/day for adults and 1250 mg for those 9 to 18 years of age due to high consumption of processed foods and cola-containing diet and regular sodas (Ilich and Kerstetter 2000). While increased phosphorus interferes with the sympathetic response of PTH and increases bone resorption, it remains likely that higher calcium intake can serve as a counterbalance (Heaney 2009).

Most phosphorus-containing foods including meat, dairy, nuts, legumes, and cereals also contain nutrients that positively impact bone health such as protein, magnesium, vitamin D, and calcium. Thus, eliminating or reducing these foods is not the issue. Rather, to positively impact bone health, female athletes should be advised to favor nutrient-dense whole foods and beverages over nutrient-poor processed foods and soda. Current data in the general population suggest that about 50% of adolescents and 65% of younger girls achieve the RDA of 1250 mg/day (Moshfegh et al. 2009). Barrack and colleagues' (2010) study on high school runners supports these findings in her group with normal bone turnover. In the group of runners experiencing elevated bone turnover, only 46% met the RDA. Perhaps more important than phosphorus intake is the fact that these same girls consume grossly inadequate amounts of calcium (Heaney 2007; Moshfegh et al. 2009; Barrack et al. 2010).

Magnesium

Magnesium is absorbed by the surface of crystals in the bone matrix (Ilich and Kerstetter 2000), and more than half of the magnesium in the body is found combined with calcium and phosphorus in the bone (Bergman et al. 2009). Research shows that low serum magnesium can impair PTH secretion and alter serum calcium levels, thus impacting bone turnover (Ilich and Kerstetter 2000). Population data suggest that higher dietary magnesium correlates with improved BMD (Ilich and Kerstetter 2000). The exact mechanism by which calcium and magnesium interact is not fully understood. Calcium intervention trials demonstrate improved bone mass with or without the addition of magnesium (Heaney 2009), so it is clear that more research is needed to determine how the two work in conjunction with one another. The key message lies in understanding the dietary patterns of female athletes. Many females, especially adolescents, as well as female athletes fall short of recommended magnesium intakes (Ilich and Kerstetter 2000; Moshfegh et al. 2009; Barrack et al. 2010). Emphasizing a nutrient-dense diet containing whole grains, vegetables, nuts, and seeds not only delivers adequate levels of magnesium but also protein, potassium, and antioxidants—nutrients that also contribute to overall bone health.

Fluoride

Fluoride is most commonly associated with tooth development and preservation and the reduction of dental caries in young children. However, it is also an important constituent of bone. In fact, 99% of the total-body fluoride is found in the skeletal system. Most dietary fluoride comes from fluoridated water, seafood, and tea. In smaller amounts fluoride is found in grains, legumes, and root vegetables; however,

amounts of the mineral can vary widely based on mineral content of irrigation water. When consumed by way of water or toothpaste, fluoride is readily and rapidly absorbed in the stomach; when consumed with foods and beverages, bioavailability may decrease as much as 50% as it binds to other minerals (Bergman et al. 2009). Once absorbed, fluoride demonstrates strong affinity for the tissues of bones and teeth. In the skeleton, fluoride is incorporated into the hydroxyapatite crystal structure of the bone matrix where it increases the crystal size, which may prevent osteoclastic attack (Ilich and Kerstetter 2000). In addition to playing a role in bone structure, fluoride improves osteoblastic activity in both cortical and trabecular bone (Resch et al. 1993).

While some fluoride is beneficial for bone, too much can be detrimental. Crystals in the bone structure that grow too large may become brittle and result in fluorosis (Ilich and Kerstetter 2000). The bone-debilitating condition is rare in the United States; however, it has been studied extensively in India and China. In 2001, Li et al. (2001) demonstrated that Chinese women consuming fluoridated water in the range of 1 to 1.06 ppm exhibited decreased overall fracture risk when compared to women consuming water with negligible amounts. At levels ≥4.32 ppm, fracture risk increased (Li et al. 2001). In the United States, fluoride in the water supply is widely variable, and while the federal government sets appropriate ranges, decisions about whether or not to fluoridate water and at what amount often fall to county jurisdiction.

Research has investigated the use of fluoride as a therapeutic agent to prevent bone loss in osteoporotic patients; however, outcomes remain mixed and its use as supplemental therapy for bone health is not supported (Ilich and Kerstetter 2000). Research specifically linking fluoride and the female athlete is scarce. Regarding this mineral, professionals should encourage dietary variety, use of fluoridated toothpaste, and normal water consumption to help female athletes meet their fluoride needs for bone health.

Iron and Zinc

Iron functions as a cofactor for enzymes involved in collagen synthesis, so its presence in the diet is essential for bone formation and remodeling. Ilich-Ernst et al. followed 354 girls (premenarcheal at baseline) for 4 years and identified a baseline association between serum ferritin and bone mineral density, suggesting that iron deficiency may play a role in bone fragility during the developmental years (Ilich-Ernst et al. 1998). As described in Chapter 5, it is not uncommon for female athletes to present with iron-deficiency anemia or low serum ferritin as a result of menstrual bleeding, poor diet, growth demands, or sport-specific stress (i.e., long-distance running), so considering iron status may be an important marker not only for assessing and treating potential anemia but also for optimal bone health. Because iron and calcium, both divalent cations, compete for intestinal absorption, it is often recommended that female athletes who consume both supplemental calcium and iron split administration between two time periods for maximal absorption. Ilich-Ernst et al. also noted that long-term calcium supplementation did not detrimentally affect iron stores (Ilich-Ernst et al. 1998), evidence that dual supplementation is possible and potentially necessary to treat multiple conditions.

Just as iron deficiency and low serum ferritin may impair bone health, so too may the pro-oxidant effects of iron overload (Ilich and Kerstetter 2000). Those with

hemochromatosis or chronic renal disease may have increased risk for osteoporosis. It is unclear, however, whether this is due to the direct effects of excessive iron or a result of hypovitaminosis C or other dietary, lifestyle, or genetic factors (Ilich and Kerstetter 2000). Regardless, because of the other potential dangers associated with excessive iron intake (see Chapter 5), female athletes should focus on consuming iron-rich foods and only supplement under the direction of a qualified health professional.

Inadequate zinc impacts bone through direct and indirect pathways. Like iron, zinc is a cofactor in collagen synthesis (Ilich and Kerstetter 2000); it is also found in enzymes essential for bone metabolism (Heaney 2009), and low levels of dietary zinc are associated with decreased BMD and stunted growth (Heaney 2009). Furthermore, zinc is required for protein metabolism. Decreased availability impairs protein function, which in turn negatively impacts bone health (Ilich and Kerstetter 2000). Although the populations at greatest risk for zinc deficiency include infants and adolescents (due to their increased growth requirements and often poor dietary habits), female athletes, especially adolescent athletes, may also be at risk. Chapter 5 details what is known regarding zinc intakes and zinc status in female athletes and provides dietary guidance for meeting zinc requirements.

Vitamin K

Epidemiological data from the Nurses Health Study cohort suggest that low dietary vitamin K is associated with increased age-adjusted relative risk for fracture in women ages 38 to 63 years. This study also observed an inverse relationship between lettuce consumption and risk of hip fracture, demonstrating the importance of food-based vitamin K (Feskanich et al. 1999). In a separate cohort, women in the Framingham Heart Study exhibited low BMD when vitamin K was low (Feskanich et al. 1999; Booth et al. 2003). Because vitamin K is integral for the proper carboxylation of osteocalcin (Heaney 2009), which is the primary noncollagenous protein incorporated in the bone matrix, it is an essential vitamin for proper bone formation (Ilich and Kerstetter 2000). Undercarboxylated osteocalcin has low biological activity and is elevated in those with osteoporosis, most likely a result of low vitamin K (Ilich and Kerstetter 2000). After adjusting for confounders, including age, Booth et al. observed that women (average age 58 ± 9 years) participating in the Framingham Heart Study in the lowest quartile of vitamin K intake (70.2 µg/day) had significantly lower bone density in the femoral neck and spine than those in the highest quartile of intake (309 µg/day). Because this study exhibited no such associations in men, there may be a sex-specific effect of vitamin K on bone (Booth et al. 2003). Barrack and colleagues' (2010) research on female runners indicated that about 40% fall short of vitamin K recommendations. The best sources of vitamin K are leafy greens and fortified oils, both of which demonstrate equal bioavailability among men and women ages 30 to 70 years (Booth et al. 1999). Because these foods may not be ubiquitous in the typical adolescent diet, care must be taken to find foods and dietary patterns that meet requirements.

In women, the effectiveness of supplemental vitamin K in reducing fracture risk remains questionable. Research among children (3 to 16 years) identified a correlation between dietary vitamin K and biochemical markers of bone turnover but could

not extrapolate this to bone mass or bone strength (Kalkwarf et al. 2004). Another study demonstrated an increase in bone strength with 45 mg/day supplemental vitamin K2* (menaquinone) compared to placebo in postmenopausal women (Knapen et al. 2007). More recent reviews note the inconsistency of the data (Cashman and O'Connor 2008; Shea and Booth 2008). Furthermore, an intervention trial evaluating the impact of vitamin K1 supplementation on bone loss in 115 female athletes found no effect among eumenorrheic, amenorrheic, or estrogen-supplemented groups (Braam et al. 2003).

With little consensus in the literature, athletes should strive for a daily intake of 90 μg/day, derived primarily from green vegetables including broccoli, kale, spinach, turnip greens, and Brussels sprouts, which all contain at least 100 μg/100 g serving. Intestinal bacteria, cultivated by a healthy diet, synthesize vitamin K2 and can help improve bone health. Generally, diets rich in vitamin K are suggestive of an overall healthy eating pattern correlated with higher calcium and vitamin D intakes.

Antioxidants

Research suggests that antioxidant-rich fruits and vegetables play a protective role in other chronic diseases; thus, it follows that they may play a role in bone health (Ilich and Kerstetter 2000). Vitamins A and C are both essential for proper bone remodeling. Vitamin C is required for collagen cross-linking, and as an antioxidant, vitamin C may protect bone against oxidative stress (Ilich and Kerstetter 2000). Similar to fluoride, vitamin A shares a biphasic relationship with bone. Both osteoblasts and osteoclasts contain nuclear receptors for retinoic acid demonstrating the importance of vitamin A in the complete remodeling process. In cases of vitamin A deficiency, osteoclast activity is too low and osteoblast activity proceeds unchecked resulting in excessive bone deposition (Ilich and Kerstetter 2000). In excess, vitamin A increases fracture risk as bone resorption accelerates. A diet rich in colorful fruits and vegetables will contribute both vitamins C and A (as β-carotene) to the diet. Dietary forms of preformed vitamin A or retinoic acid include animal products, especially organ meats, and high levels can be toxic.

While it is not difficult for females to meet vitamin C recommendations (65 mg/day for adolescents and 75 mg/day for adults), and most data suggest adequate intake among a female athlete population, there is some research to suggest that certain groups may be vulnerable to poor intake (Barrack et al. 2010). Less is known about vitamin A intakes of female athletes; however, given the ubiquitous food sources of beta carotene, and the similarities between those food sources and vitamin C–containing foods, it is unlikely to see widespread deficiency. Again, it is important to be cognizant of dietary avoidances, limited food intake, and sport-induced demands when performing assessments and making recommendations.

* Vitamin K exists in two primary forms: K1, phylloquinone, is found predominately in green leafy vegetables; K2, menaquinone, has many forms and is derived from bacterial fermentation. It forms naturally in the gut and is found in some fermented foods.

Dietary Components That May Negatively Impact Bone Health

Sodium

Sodium is often cited as a nutrient that negatively impacts bone health due to the well-established relationship between increased sodium consumption and the resultant increase in urinary calcium excretion (Heaney 2009; Ilich and Kerstetter 2000); however, much of this evidence comes from research examining the impacts of acute salt loading (Teucher and Fairweather-Tait 2003). Few studies have sought to examine the impact of long-term sodium intake on bone health. A 2-year longitudinal study evaluated bone density in 124 postmenopausal women and demonstrated that high sodium intake negatively impacted BMD at the hip (Devine et al. 1995). A more recent study of 16 postmenopausal women examined the impact of varying salt and calcium intakes on calcium balance revealing that at low calcium intakes (518 mg/day) bone balance is negative at both low and high levels of dietary sodium. When calcium intake is higher (1284 mg/day), bone balance is positive at low sodium levels (1557 mg/day) and negative when sodium intake is high (4422 mg/day) (Teucher et al. 2008). Thus, we can conclude that both calcium and sodium impact bone; adequate calcium is essential, and excessive sodium can be detrimental to bone health. One complicating factor, however, is that differences in salt sensitivity impact how dietary sodium impacts calciuria, so results are not always predictable due to inter-individual variability (Teucher et al. 2008).

In this area, little is known about the specific relationship between sodium, bone health, and female athletes, and it is difficult to directly apply the data from postmenopausal women to female athletes. Because of exercise-induced sodium demands, it is unlikely that female athletes are at risk of bone damage due to increased sodium intake; however, dietary patterns associated with higher sodium intakes should not be overlooked. The typical Western diet is high in sodium, which negatively correlates with potassium and calcium intake. When making dietary recommendations, it would be prudent to emphasize potassium-rich foods, found primarily in whole foods (fruits, vegetables, and dairy). Potassium works in opposition to sodium by decreasing urinary calcium excretion, and an increase in dietary potassium may help protect against calcium losses (Heaney 2009).

Carbonated Beverages

Observational research has associated carbonated beverage consumption, specifically cola-type beverages, with increased fracture risk in high school girls (Braam et al. 2003). In one particular survey-based study of 490 ninth- and tenth-grade students, physically active girls who consumed both cola and non-cola-type beverages had the greatest fracture risk (Wyshak 2000). This study did not discuss possible mechanisms of action. In older women from the Framingham Heart Cohort (average age 58 ± 9 years), Tucker et al. observed low BMD among women consuming cola-type carbonated beverages but not with non-cola varieties—this observation remained after adjusting for caffeine intake (Tucker et al. 2006). Additional research further indicates that this correlation is unlikely to be attributable to the beverage's caffeine, phosphorus, or carbonation because any increase in urinary calcium excretion caused by caffeine or phosphoric acid is negligible (Heaney and Rafferty 2001).

Plausible explanations for these observations are that high consumption of carbonated beverages may displace calcium-containing milk and dairy products or that diets rich in cola-containing carbonated beverages are indicative of poor overall diet quality, low overall calcium intake, and possible deleterious effects of excessive phosphoric acid on calcium absorption (Wyshak 2000; Tucker et al. 2006). Research is still needed to examine the specific effects of carbonated-beverage intake on bone health, and greater attention must be paid to overall diet quality and lifestyle patterns with a focus on maximizing those elements known to promote bone health.

Caffeine

Despite the anecdotal allegations, research shows that caffeine has minimal impact on calcium overall balance, and the addition of a small amount of dairy (e.g., adding 2 Tbsp milk to a 6 oz cup of coffee) more than offsets any increase in urinary calcium excretion (Ilich and Kerstetter 2000). Furthermore, an immediate rise in calcium excretion due to caffeine ingestion is offset by decreased calciuria later in the day (Heaney and Rafferty 2001). As long as calcium intake is adequate, moderate amounts of dietary caffeine are not problematic for bone health. In young female athletes, calcium intake is often inadequate; however, caffeine intake has also been reported as low (Ihle and Loucks 2004). Yet we see an increasing number of caffeine-containing sport nutrition products on the market (e.g., energy drinks, gels, and blocks), many of which have ad campaigns directed toward young athletes. Clinicians should be aware of the potential caffeine boost these products may deliver and screen for intake when working with individual athletes or groups. Furthermore, considering the known benefit of caffeine as an ergogenic aid, sport dietitians should take care when making recommendations to *young* female athletes. Ensuring adequate calcium intake is integral for bone protection.

Alcohol

In moderation, alcohol is positively associated with bone health in premenopausal women (Ilich and Kerstetter 2000; Macdonald 2009). Perhaps moderate consumption is indicative of a healthier overall dietary pattern or perhaps it is alcohol's contribution of beneficial flavonoids and antioxidants that illicit this positive impact. In a postmenopausal population, alcohol stimulates androstenedione conversion into estrone, providing this estrogen-deficient group with a small amount of the bone-protective hormone (Ilich and Kerstetter 2000). Regardless, excessive consumption is extremely detrimental to bone health. High alcohol consumption is often indicative of subsequent problems and can be associated with poor diet and malabsorption of important bone nutrients including calcium, magnesium, iron, and zinc. Often a result of excessive alcohol consumption, liver disease disrupts endocrine function including vitamin D and PTH, and too much alcohol is directly toxic to osteoblasts and associated with increased risk for falls (Ilich and Kerstetter 2000). Female athletes should take care. Excessive alcohol consumption may not only result in essential nutrient malabsorption, but it may also displace other nutrients important for bone health (i.e., protein, calcium) and result in dehydration. College represents an opportunity for alcohol consumption, and research shows that female athletes are not exempt from this group. In fact, college athletes may be more likely to report heavy episodic drinking (Martens et al. 2011).

The health professional should be aware of these trends as well as the negative impact excessive alcohol can have on the health and performance of female athletes.

Dietary Supplements Targeted toward Bone Health

While food sources remain the best source of bioavailable bone-enhancing nutrients, there are cases in which supplementation may be a necessary adjunct therapy. The majority of supplements available combine calcium with vitamin D. Some products include other bone-building nutrients, such as magnesium or zinc, but all make claims to improve bone health. With respect to supplemental calcium, the most widely available sources and best in terms of bioavailability are calcium carbonate and calcium citrate. Calcium carbonate contains more elemental calcium than calcium citrate (40% versus 21%, respectively) and may require fewer pills per dose. This could make calcium carbonate a better choice for those with aversions to swallowing multiple pills; however, when comparing the two options, tolerance reports demonstrate a greater association of gastric upset (i.e., bloating, constipation, flatulence) with calcium carbonate. To limit this, calcium carbonate is best taken in an acidic environment, with food. For those taking calcium supplements on an empty stomach and for those with acid reflux conditions, calcium citrate can be taken without food and may be a better option (Ross and U.S. Institute of Medicine 2011). This information may help nutrition and health professionals offer best practice recommendations for female athletes based on individual preferences and medical history.

Other options available include calcium that is chelated to other salts (i.e., calcium gluconate, calcium lactate, calcium phosphate); however, research does not support their use over more widely available, less expensive options. Calcium absorption can be influenced by several dietary factors, and calcium may inhibit absorption of other key nutrients (zinc, iron, magnesium); thus, taking calcium supplements between meals for those who are also taking supplemental doses of other micronutrients may be warranted. A quick scan of the supplement shelf at the pharmacy will reveal the multitude of products available in this area. Many combination supplements include calcium, magnesium, and zinc in their formulation. While all three minerals are important for bone health, they compete for intestinal absorption, so when taken in combination, total absorption will likely decline. For female athletes, additional supplementation with magnesium and zinc should be evaluated on a case-by-case basis, and food-based sources are preferred. For optimal absorption, calcium with vitamin D is the best option. When considering calcium supplementation, it is important to keep in mind that absorption is best when broken into 500 mg doses. As discussed previously, vitamin D supplementation may be warranted, especially in winter months. Current wisdom suggests daily doses of 800 to 2000 IU (Willis et al. 2008). Although supplements with both vitamin D_2 and D_3 are available, D_2 is only about 30% as effective at maintaining 25(OH)D levels (Holick 2007), so recommendations for the female athlete should include vitamin D_3 (cholecalciferol). When working with athletes, an assessment of current eating habits and practices will help determine food-based dietary calcium and guide supplement recommendations.

CONCLUSION

A number of nutrients are important for bone health in female athletes, but no single food or nutrient can take the place of calories; adequate energy availability is imperative for maintaining skeletal health. Emerging research continues to elucidate the relationship between energy availability and bone metabolism, and new discoveries have identified leptin as a key modulator. Low energy availability negatively impacts reproductive hormones and osteoblast cell differentiation, both of which are important players in the game of optimizing bone health. Of course, in addition to the calorie content of the diet, attention must be given to the quality of those calories. Female athletes should favor nutrient-dense macronutrients that will contribute valuable bone-building micronutrients. High-quality dietary protein, from both animal and vegetable sources, is essential for healthy bone remodeling. Female athletes require more protein than their nonathletic counterparts, and current research does not support the acid-ash hypothesis, stating that too much protein promotes calcium loss. Increased dietary protein may increase calciuria, but it also boosts calcium absorption and positively impacts endocrine markers for bone health. Likewise, female athletes should not shy away from the incorporation of dietary fat. While specific research linking dietary fat, the female athlete, and bone health remains scarce, we do know that omega-3 fatty acids may help reduce inflammation, and that athletes with too little fat stores exhibit fat-regulated endocrine dysfunction.

The minerals calcium, phosphorus, magnesium, and fluoride all contribute to bone's crystalline architecture, and vitamins D, K, A, and C contribute to maintaining an appropriately coupled relationship between bone formation and bone resorption. Food-based sources are always the preferred method of introducing and assimilating these nutrients into the diet. As we are continuing to discover, nutrients work in synergy with one another, and many whole foods contain several of the bone-building nutrients as well as other compounds that promote both overall health and bone health. Attention to diet throughout the athlete's life cycle is important for maintaining a lifetime of bone health; however, vigilance during the prepubertal and adolescent years, when bone is accruing most rapidly, will help athletes reach their genetically programmed PBM and reduce risk for fracture later in life. Focusing on the overall dietary patterns of athletes to emphasize a high-quality diet will ensure an eating pattern rich in all of the bone-building macro- and micronutrients.

REFERENCES

Aloia, J. F., et al., Serum vitamin D metabolites and intestinal calcium absorption efficiency in women. *Am J Clin Nutr*, 2010. 92(4): 835–840.

Barrack, M. T., et al., Physiologic and behavioral indicators of energy deficiency in female adolescent runners with elevated bone turnover. *Am J Clin Nutr*, 2010. 92(3): 652–659.

Beasley, J. M., et al., Is protein intake associated with bone mineral density in young women? *Am J Clin Nutr*, 2010. 91(5): 1311–1316.

Bergman, C., et al., What is next for the Dietary Reference Intakes for bone metabolism related nutrients beyond calcium: Phosphorus, magnesium, vitamin D, and fluoride? *Crit Rev Food Sci Nutr*, 2009. 49(2): 136–144.

Bonjour, J. P., Dietary protein: An essential nutrient for bone health. *J Am Coll Nutr*, 2005. 24(Suppl. 6): 526S–536S.

Booth, S. L., et al., Response of vitamin K status to different intakes and sources of phylloquinone-rich foods: Comparison of younger and older adults. *Am J Clin Nutr*, 1999. 70(3): 368–377.

Booth, S. L., et al., Vitamin K intake and bone mineral density in women and men. *Am J Clin Nutr*, 2003. 77(2): 512–516.

Braam, L. A., et al., Factors affecting bone loss in female endurance athletes: A two-year follow-up study. *Am J Sports Med*, 2003. 31(6): 889–895.

Cannell, J. J., et al., Epidemic influenza and vitamin D. *Epidemiol Infect*, 2006. 134(6): 1129–1140.

Cannell, J. J., et al., Athletic performance and vitamin D. *Med Sci Sports Exerc*, 2009. 41(5): 1102–1110.

Cao, J. J., and F. H. Nielsen, Acid diet (high-meat protein) effects on calcium metabolism and bone health. *Curr Opin Clin Nutr Metab Care*, 2010. 13(6): 698–702.

Cashman, K. D., and E. O'Connor, Does high vitamin K1 intake protect against bone loss in later life? *Nutr Rev*, 2008. 66(9): 532–538.

Confavreux, C. B., Bone: From a reservoir of minerals to a regulator of energy metabolism. *Kidney Int*, 2011.

Constantini, N. W., et al., High prevalence of vitamin D insufficiency in athletes and dancers. *Clin J Sport Med*, 2010. 20(5): 368–371.

Darling, A. L., et al., Dietary protein and bone health: A systematic review and meta-analysis. *Am J Clin Nutr*, 2009. 90(6): 1674–1692.

De Souza, M. J., et al., The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone*, 2008. 43(1): 140–148.

Devine, A., et al., A longitudinal study of the effect of sodium and calcium intakes on regional bone density in postmenopausal women. *Am J Clin Nutr*, 1995. 62(4): 740–745.

Fenton, T. R., et al., Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance. *J Bone Miner Res*, 2009. 24(11): 1835–1840.

Feskanich, D., et al., Vitamin K intake and hip fractures in women: A prospective study. *Am J Clin Nutr*, 1999. 69(1): 74–79.

Gordon, C. M., Clinical practice. Functional hypothalamic amenorrhea. *N Engl J Med*, 2010. 363(4): 365–371.

Greydanus, D. E., H. Omar, and H. D. Pratt, The adolescent female athlete: Current concepts and conundrums. *Pediatr Clin North Am*, 2010. 57(3): 697–718.

Halliday, T. M., et al., Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Med Sci Sports Exerc*, 2011. 43(2): 335–343.

Heaney, R. P., The bone remodeling transient: Interpreting interventions involving bone-related nutrients. *Nutr Rev*, 2001. 59(10): 327–334.

Heaney, R. P., Bone health. *Am J Clin Nutr*, 2007. 85(1): 300S–303S.

Heaney, R. P., Dairy and bone health. *J Am Coll Nutr*, 2009. 28 Suppl 1: 82S–90S.

Heaney, R. P., and M. F. Holick, Why the IOM recommendations for vitamin D are deficient. *J Bone Miner Res*, 2011. 26(3): 455–457.

Heaney, R. P., and K. Rafferty, Carbonated beverages and urinary calcium excretion. *Am J Clin Nutr*, 2001. 74(3): 343–347.

Hind, K., and M. Burrows, Weight-bearing exercise and bone mineral accrual in children and adolescents: A review of controlled trials. *Bone*, 2007. 40(1): 14–27.

Holick, M. F., Vitamin D deficiency. *N Engl J Med*, 2007. 357(3): 266–281.

Hunt, J. R., L. K. Johnson, and Z. K. Fariba Roughead, Dietary protein and calcium interact to influence calcium retention: A controlled feeding study. *Am J Clin Nutr*, 2009. 89(5): 1357–1365.

Ihle, R., and A. B. Loucks, Dose-response relationships between energy availability and bone turnover in young exercising women. *J Bone Miner Res*, 2004. 19(8): 1231–1240.

Ilich, J. Z., and J. E. Kerstetter, Nutrition in bone health revisited: A story beyond calcium. *J Am Coll Nutr*, 2000. 19(6): 715–737.

Ilich-Ernst, J. Z., et al., Iron status, menarche, and calcium supplementation in adolescent girls. *Am J Clin Nutr*, 1998. 68(4): 880–887.

Kalkwarf, H. J., et al., Vitamin K, bone turnover, and bone mass in girls. *Am J Clin Nutr*, 2004. 80(4): 1075–1080.

Karsenty, G., Convergence between bone and energy homeostases: Leptin regulation of bone mass. *Cell Metab*, 2006. 4(5): 341–348.

Kerstetter, J. E., et al., The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *J Clin Endocrinol Metab*, 2005. 90(1): 26–31.

Knapen, M. H., L. J. Schurgers, and C. Vermeer, Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int*, 2007. 18(7): 963–972.

Lacativa, P. G. and M. L. Farias, Osteoporosis and inflammation. *Arq Bras Endocrinol Metabol,* 2010. 54(2): 123–132.

Lappe, J., et al., Calcium and vitamin D supplementation decreases incidence of stress fractures in female navy recruits. *J Bone Miner Res*, 2008. 23(5): 741–749.

Larson-Meyer, D. E., and K. S. Willis, Vitamin D and athletes. *Curr Sports Med Rep*, 2010. 9(4): 220–226.

Li, Y., et al., Effect of long-term exposure to fluoride in drinking water on risks of bone fractures. *J Bone Miner Res*, 2001. 16(5): 932–939.

Macdonald, H. M., Alcohol and recommendations for bone health: Should we still exercise caution? *Am J Clin Nutr*, 2009. 89(4): 999–1000.

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

Marieb, E. N., *Human anatomy and physiology*, 6th ed., 2004, New York: Pearson Education, xxi, 1242.

Martens, M. P., et al., Predictors of alcohol-related outcomes in college athletes: The roles of trait urgency and drinking motives. *Addict Behav*, 2011. 36(5): 456–464.

Matkovic, V., et al., Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. Inference from a cross-sectional model. *J Clin Invest*, 1994. 93(2): 799–808.

Moshfegh, A., G. Joseph, J. Ahuja, D. Rhodes, and R. LaComb, What We Eat in America, NHANES 2005–2006: Usual Nutrient Intakes from Food and Water Compared to 1997 Dietary Reference Intakes for Vitamin D, Calcium, Phosphorus, and Magnesium. A.R.S. U.S. Department of Agriculture, Editor. 2009.

Nattiv, A., et al., American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc*, 2007. 39(10): 1867–1882.

Resch, H., et al., Evidence that fluoride therapy increases trabecular bone density in a peripheral skeletal site. *J Clin Endocrinol Metab*, 1993. 76(6): 1622–1624.

Rosen, C. J., Bone remodeling, energy metabolism, and the molecular clock. *Cell Metab*, 2008. 7(1): 7–10.

Ross, A. C., and U.S. Institute of Medicine. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary reference intakes for calcium and vitamin D. 2011, Washington, DC: National Academies Press.

Roughead, Z. K., et al., Controlled substitution of soy protein for meat protein: Effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women. *J Clin Endocrinol Metab*, 2005. 90(1): 181–189.

Ruohola, J. P., et al., Association between serum 25(OH)D concentrations and bone stress fractures in Finnish young men. *J Bone Miner Res*, 2006. 21(9): 1483–1488.

Russell, M., and M. Misra, Influence of ghrelin and adipocytokines on bone mineral density in adolescent female athletes with amenorrhea and eumenorrheic athletes. *Med Sport Sci*, 2010. 55: 103–113.

Salari, P., et al., A systematic review of the impact of n-3 fatty acids in bone health and osteoporosis. *Med Sci Monit*, 2008. 14(3): RA37–RA44.

Shea, M. K., and S. L. Booth, Update on the role of vitamin K in skeletal health. *Nutr Rev*, 2008. 66(10): 549–557.

Teucher, B., and S. Fairweather-Tait, Dietary sodium as a risk factor for osteoporosis: Where is the evidence? *Proc Nutr Soc*, 2003. 62(4): 859–866.

Teucher, B., et al., Sodium and bone health: Impact of moderately high and low salt intakes on calcium metabolism in postmenopausal women. *J Bone Miner Res*, 2008. 23(9): 1477–1485.

Tucker, K. L., et al., Colas, but not other carbonated beverages, are associated with low bone mineral density in older women: The Framingham Osteoporosis Study. *Am J Clin Nutr,* 2006. 84(4): 936–942.

Weikert, C., et al., The relation between dietary protein, calcium and bone health in women: Results from the EPIC-Potsdam cohort. *Ann Nutr Metab*, 2005. 49(5): 312–318.

Willis, K. S., N. J. Peterson, and D. E. Larson-Meyer, Should we be concerned about the vitamin D status of athletes? *Int J Sport Nutr Exerc Metab*, 2008. 18(2): 204–224.

Wyshak, G., Teenaged girls, carbonated beverage consumption, and bone fractures. *Arch Pediatr Adolesc Med*, 2000. 154(6): 610–613.

7 B Vitamins and the Female Athlete

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CONTENTS

INTRODUCTION

The B-vitamins play important functions in maintaining the health of female athletes and active women (Maughan 1999; Manore 1999, 2000; Lukaski 2004; Wildman and Miller 2004; Woolf and Manore 2006, 2007; Volpe 2007; Manore et al. 2009). Thiamin, riboflavin, niacin, vitamin B6, pantothenic acid, biotin, and choline function as coenzymes in the energy-producing pathways of the body, while folate and vitamin B_{12} are required for the production of healthy red blood cells and cell regeneration.

Regular physical activity may alter the need or requirement for many nutrients, including the B vitamins (Maughan 1999; Manore 1999, 2000; Lukaski 2004; Wildman and Miller 2004; Woolf and Manore 2006, 2007; Volpe 2007; Manore et al. 2009). First, the metabolic pathways used to produce energy are stressed during physical activity; thus, requirements for nutrients used in these pathways may be increased. Second, biochemical adaptations in the tissues of the body which occur with training may increase requirements of these nutrients. Third, regular physical activity may also increase the turnover or loss of nutrients in the sweat, urine, or feces. Finally, additional nutrients may be required to repair and maintain the higher lean tissue mass of the active individual.

When the Dietary Reference Intakes (DRIs) for the B-vitamins were established, information was not available to determine specific recommendations for active or athletic women (Food and Nutrition Board 1998). If exercise does in fact increase the need for the B-vitamins, then female athletes just meeting dietary recommendations may exhibit poor nutrient status. Additionally, female athletes may be at risk of poor nutrient status if they purposefully restrict energy intake, due to dieting and body image concerns, and thus reduce micronutrient intakes (Hawley et al. 1995; Manore 1999; Beals and Manore 2007). Furthermore, due to revised dietary recommendations and criteria of adequacy, it is challenging to historically assess B-vitamin intake and status across studies.

This chapter will review the exercise-related functions, food sources, and recommended intakes for each of the B-vitamins. The research examining nutritional status (intake and biochemical markers) and exercise performance for the female athlete will also be discussed.

THIAMIN

Exercise-Related Functions

Thiamin is important for the metabolism of carbohydrates, fat, and branched chain amino acids (Table 7.1) (Maughan 1999; Manore 1999, 2000; Manore et al. 2000, 2009; Lukaski 2004; Wildman and Miller 2004; Woolf and Manore 2006, 2007; Volpe 2007). For example, thiamin serves as a coenzyme for a number of metabolic reactions, including the oxidative decarboxylation of pyruvate to acetyl CoA, the formation of succinyl CoA during one of the steps of the tricarboxylic acid (TCA) cycle, and the metabolism of branched-chain amino acids. Thiamin is also needed in the pentose phosophate pathway and for the functioning of neural tissue. Because exercising muscle depends on the metabolism of these nutrients, thiamin is critical for energy metabolism and athletic performance.

Food Sources and Recommended Intakes

Thiamin is found in a variety of foods, such as whole grains and enriched-grain products, meats (especially pork), liver, nuts, and green leafy vegetables (Table 7.2). The 1998 Estimated Average Requirement (EAR) for thiamin is 1.0 and 0.9 mg/day for adult men and women, respectively, while the Recommended Dietary Allowance (RDA) is 1.2 and 1.1 mg/day for adult men and women, respectively (Food and Nutrition Board 1998) (Table 7.2). Thiamin does not have a tolerable upper intake level (UL).

Assessment of Status

Status of thiamin can be assessed by measuring thiamin in the blood or urine (Warnock et al. 1979; Finglas 1994). Because urinary thiamin excretion decreases with poor thiamin intakes, urinary thiamin excretion <40 μg/day suggests poor status (Bayliss et al. 1984; Gibson 2005). Transketolase, a thiamin-dependent enzyme of the pentose phosphate pathway that produces NADPH and ribose-phosphate, can

TABLE 7.1
Exercise-Related Metabolic Functions of the B-Complex Vitamins

Vitamin	Active Form	Exercise-Related Functions
Thiamin	Thiamin pyrophosphate (TPP)	Serves as a coenzyme in reactions in the energy pathways: • Pentose phosphate pathway (transketolase) • Oxidative decarboxylation of pyruvate to acetyl CoA (pyruvate dehydrogenase) • Tricarboxylic acid (TCA) cycle (α-ketoglutarate dehydrogenase) • Branched-chain amino acid metabolism (branched-chain α-keto acid dehydrogenase complex) Assists in membrane and nerve conduction
Riboflavin	Flavin mononucleotide (FMN); flavin adenine dinucleotide (FAD)	Serves as a coenzyme in oxidation-reduction reactions in energy pathways: • TCA cycle (succinate dehydrogenase) • Oxidative decarboxylation of pyruvate to acetyl CoA (pyruvate dehydrogenase) • Fatty acid β-oxidation (acyl-CoA dehydrogenase) Aids in conversion of folate and vitamin B_6 to their active forms Aids in synthesis of niacin from tryptophan
Niacin, nicotinic acid	Nicotinamide adenine dinucleotide (NAD); nicotinamide adenine dinucleotide phosphate (NADP)	Serves as a coenzyme in oxidation-reduction reactions in metabolic pathways: • Glycolysis (glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase) • Oxidative decarboxylation of pyruvate to acetyl CoA (pyruvate dehydrogenase) • TCA cycle (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase) • Pentose phosphate pathway (glucose-6-phosphate dehydrogenase) • Oxidative deamination of amino acids (glutamate dehydrogenase) • Fatty acid biosynthesis (fatty acid synthase) and β-oxidation (3-hydroxyacyl CoA dehydrogenase)
Vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine)	Pyridoxal phosphate (PLP)	Serves as a coenzyme in amino acid metabolism: • Transaminase reactions (aspartic amino transferase [AST], alanine aminotransferase [ALT]) • Deamination/dehydration reactions (threonine dehydratase) • Niacin synthesis from tryptophan Serves as coenzyme in glycogen breakdown (glycogen phosphorylase)
Pantothenic acid	Coenzyme A (CoA); acyl carrier protein (ACP)	Functions as a component of two compounds involved in energy metabolism: • Coenzyme A (activates compounds allowing them to be metabolized [acetyl CoA, succinyl CoA, propionyl CoA, malonyl CoA]) • Acyl carrier protein (preliminary step in fatty acid synthesis)

TABLE 7.1 ***(Continued)***
Exercise-Related Metabolic Functions of the B-Complex Vitamins

Vitamin	Active Form	Exercise-Related Functions
Biotin	Biotin	Serves as a coenzyme in the metabolism of carbohydrates, fatty acids, and amino acids: • Fatty acid biosynthesis (acetyl CoA carboxylase) • Conversion of pyruvate to oxaloacetate to maintain TCA cycle intermediates and for gluconeogenesis (pyruvate carboxylase) • Oxidation of odd-chain-length fatty acids and metabolism of isoleucine, threonine, and methionine (propionyl CoA carboxylase) • Metabolism of leucine (β-methylcrotonyl CoA carboxylase)
Choline	Choline	Functions as a component of phosphatidylcholine, the main phospholipid in cell membranes (lecithin), and acetylcholine, the neurotransmitter at neuromuscular junctions Plays a role in methyl-group metabolism
Folate (food form), folic acid (synthetic form)	Tetrahydrofolate (THF)	Functions as a coenzyme in reactions that involve: • Deoxyribonucleic acid (DNA) synthesis (methylation of deoxyuridylic acid to thymidylic acid) • Purine synthesis • Amino acid metabolism (histidine to glutamic acid, glycine to serine, and homocysteine to methionine) • Red blood cell production (conversion of megaloblasts into mature red blood cells)
Vitamin B_{12}	Cobalamin	Serves as a coenzyme in the methyl transfer reaction that converts homocysteine to methionine and recycles folate (methionine synthase) Functions as coenzyme for breakdown of odd-chain-length fatty acids (methylmalonyl CoA mutase) Maintains neural tissue

be used as a functional marker of thiamin. With poor status, activity of the enzyme increases with the addition of thiamin to the assay. An increase in transketolase activity of >25% indicates thiamin deficiency (Food and Nutrition Board 1998).

Dietary Intake and Status of Thiamin in Active Adults

Although thiamin intakes by female athletes are typically less than male athletes (due to the higher energy consumption in active men), most studies report adequate dietary intakes for thiamin in female athletes (Table 7.3) (Nieman et al. 1989; Worme et al. 1990; Faber and Benadé 1991; Fogelholm et al. 1992; Manore 2000; Woolf and Manore 2006, 2007). A notable exception was a study conducted by Leydon and Wall (2002), which reported dietary intakes for thiamin less than the 1998 EAR; the male jockeys consumed less thiamin than the female jockeys (males: 0.91 ± 0.42 mg/day; females: 1.07 ± 1.08 mg/day).

TABLE 7.2
Dietary Reference Intakes for the B-Complex Vitamins for Adults Ages 19 to 50 Years

Nutrient	Male RDA[a]/EAR[b]	Female RDA[a]/EAR[b]	Male AI[c]	Female AI[c]	UL[d]	Adverse Effects of High Doses	Food Sources
Thiamin (mg/day)	1.2/1.0	1.1/0.9	—	—	ND[e]	Data are inadequate to assess risk	Whole grains and enriched-grain products, meats (especially pork), liver, dark green vegetables, nuts
Riboflavin (mg/day)	1.3/1.1	1.1/0.9	—	—	ND[e]	Data are inadequate to assess risk	Milk and dairy products, organ meats, whole grains and enriched-grain products, eggs, green leafy vegetables, and nuts
Niacin, nicotinic acid (mg/day)	16/12	14/11	—	—	35[f]	Vasodilatory effects including flushing, redness, itching, and headaches; liver injury; gastrointestinal problems	Brewer's yeast, fish, pork, beef, poultry, mushrooms, nuts, legumes, whole grains, enriched-grain products, fortified cereals, and potatoes; can also be made from the metabolism of the amino acid tryptophan
Vitamin B_6 (mg/day)	1.3/1.1	1.3/1.1	—	—	100	No adverse effect of ↑ food B_6; ↑ supplemental B_6 can cause sensory neuropathy (difficulty walking)	Animal foods, such as meat, fish, and poultry, whole grain products, noncitrus fruits and juices, and fortified cereals
Pantothenic acid (mg/day)	—	—	5	5	ND[e]	Data are inadequate to assess risk	Liver, meat, fish, poultry, milk, eggs, whole-grain products, oat cereals, potatoes, broccoli, yeast, and legumes; widely distributed in foods

Biotin (μg/day)	—	—	30	30	ND[e]	Data are inadequate to assess risk	Liver, whole-grain cereals, wheat bran, brewer's yeast, nuts, and legumes; widely distributed in foods
Choline (μg/day)	—	—	550	425	3500	Abdominal discomfort, nausea or vomiting, diarrhea, feeling of faintness or dizziness, low blood pressure, increased salivation, depression, sweating, and fishy body odor	Milk, liver, eggs, soybeans, legumes, nuts, seeds, and wheat germ
Folate (μg/day)	400/320	400/320	—	—	1000[f]	Can mask vitamin B_{12} deficiency	Fortified cereals, vegetables, whole-grain products, citrus fruits and juices, and organ meats
Vitamin B_{12} (μg/day)	2.4/2.0	2.4/2.0	—	—	ND[e]	Data are inadequate to assess risk	Meat, poultry, fish, shellfish, milk, and fortified cereals; only found naturally in animal products

[a] RDA, Recommended Dietary Allowance.
[b] EAR, Estimated Average Requirement.
[c] AI, Adequate Intake.
[d] Tolerable Upper Intake Level.
[e] ND, Not derived due to lack of data.
[f] The ULs for niacin and folate apply to synthetic forms obtained from supplements and fortified foods.

TABLE 7.3
Summary of Studies Examining Thiamin Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Beals and Manore 1998	24 female athletes with subclinical eating disorders; 24 female control athletes	7-day weighed food record	Athletes with subclinical eating disorders: 1.9 ± 0.6 mg/day Control athletes: 2.2 ± 0.6 mg/day
Beshgetoor and Nichols 2003	25 female nonsupplementing master cyclists and runners	4-day food record	2.0 ± 0.5 mg/day
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 1.5 ± 0.6 mg/day Postseason: 1.0 ± 0.4 mg/day
Faber and Benadé 1991	30 field athletes (discus, hammer, and javelin throwers; shotputters) (10 female)	7-day food record	Female: 1.31 ± 0.21 mg/day
Fogelholm et al. 1992	17 Nordic skiers (8 female)	Four 7-day food records; ETKAC	Dietary Female: 2.2 ± 0.2 mg/day ETKAC Female: 1.11 ± 0.04
Fogelholm et al. 1993	42 physically active college students (18 female)	Blood ETKAC	Female: 1.15

Kaiserauer et al. 1989	17 female distance runners (8 with amenorrhea; 9 with eumenorrhea)	3-day food record	Amenorrheic distance runners: 0.8 mg/day (mean) Eumenorrheic distance runners: 1.4 mg/day (mean)
Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	1.7 ± 1.1 mg/day
Kopp-Woodroffe et al. 1999	4 female amenorrheic runners	7-day weighed food record	1.4 ± 0.3 mg/day
Leydon and Wall 2002	19 jockeys (14 female)	7-day weighed food record	Female: 1.07 ± 1.08 mg/day
Nieman et al. 1989	347 marathon runners (56 female)	3-day food record	Female: 1.39 ± 0.60 mg/day
Sato et al. 2011	19 college swimmers (13 female)	3-day food record; blood thiamin	Dietary Female Preparation Period: 1.51 ± 0.79 mg/day Female Intensive-Training Period: 1.28 ± 0.34 mg/day Blood Female Preparation Period: 38 ± 10 ng/ml Female Intensive-Training Period: 31 ± 5 ng/ml
Worme et al. 1990	71 triathletes (21 female)	3-day food record	Female: 1.7 ± 0.5 mg/day

Source: Adapted from Woolf, K., and Manore, M. M., 2006, B Vitamins and Exercise: Does Exercise Alter Requirement, *International Journal of Sport Nutrition and Exercise Metabolism* 16: 453–484. With permission.

[a] Values reported as mean ± standard deviation unless noted.

Although research examining thiamin status in female athletes is limited, most studies with male athletes suggest that athletes with sufficient thiamin intake exhibit good status (Rankinen et al. 1998). Similar results are expected in female athletes and active women. For instance, Fogelholm (1992) assessed thiamin status before and after a 24-week exercise program in female college students (18 to 33 years). No changes were seen in biochemical markers of thiamin after the 24-week exercise program. Raczynski and Szczepanska (1993) examined thiamin status in elite Polish athletes (n = 1918; males and females combined) from 1987 to 1992. They reported that the risk of thiamin deficiency was only 2% in all athletes, with a range of 0 to 3% over this 6-year period. However, Fogelholm et al. (1993) reported different findings when they found that 12% of physically active male and female college students exhibited poor thiamin status. Unfortunately, dietary intakes were not included in this study.

Thiamin and Performance

There is no published research documenting the effects of a thiamin deficiency in female athletes; however, research in men suggests that athletic performance can be significantly reduced by thiamin deficiency (van der Beek et al. 1994). For 11 weeks, healthy men were fed a diet low in thiamin, riboflavin, and vitamin B_6. A significant decrease in aerobic power, onset of blood lactate accumulation, and peak power was observed in the nutrient-deficient men.

Like other B-vitamins, supplementation of thiamin has been promoted as a way to improve exercise performance, despite a lack of research demonstrating a positive effect on performance. Using a randomized, crossover design, Webster et al. (1997) found that a thiamin supplement taken for 4 days did not influence high-intensity exercise performance on a cycle ergometer in recreationally trained men and women. In a similar study, Webster (1998) provided highly trained cyclists (male and female) with a thiamin and pantothenic acid supplement or placebo to consume daily for 1 week prior to completing two exercise tests (50-km ride on a cycle ergometer followed by a 2000-m time trial). There were no significant differences between the two treatments for the physiological or performance outcome measures. Similarly, a thiamin supplement provided for 5 days did not enhance muscle performance in recreationally active college students (male and female) (Doyle et al. 1997). Thus, research to date seems to suggest that thiamin supplementation has no impact on exercise performance in well-nourished athletes.

RIBOFLAVIN

Exercise-Related Functions

Riboflavin serves as a coenzyme in oxidation-reduction reactions for the energy pathways in the body (Table 7.1) (Maughan 1999; Manore 1999, 2000; Manore et al. 2000, 2009; Lukaski 2004; Wildman and Miller 2004; Woolf and Manore 2006, 2007; Volpe 2007). As a component of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), riboflavin assists in the transfer of electrons from energy

pathways to the electron transport chain, essential in aerobic exercise. For instance, riboflavin is required for the oxidative decarboxylation of pyruvate to acetyl CoA, during one of the steps of the TCA cycle, and in the β-oxidation of fatty acids. Because athletes require energy from these metabolic pathways to fuel their sport, riboflavin is crucial for physical activity. Riboflavin is also required for the metabolism of other B-vitamins (folate, vitamin B_6, and niacin) (Table 7.1).

Food Sources and Recommended Intakes

Dairy products (e.g., milk, yogurt, cheese) are the most prevalent source of riboflavin in the diet of Americans (Table 7.2). Other significant sources include organ meats, eggs, whole-grain and enriched-grain products, lean meats, leafy vegetables, and nuts. The 1998 EAR for riboflavin is 1.1 and 0.9 mg/day for adult men and women, respectively, while the RDA for adult men and women is 1.3 and 1.1 mg/day, respectively (Food and Nutrition Board 1998) (Table 7.2). Riboflavin does not have a UL; however, high intakes can cause the urine to become dark yellow, which could interfere with using urine color as a marker of hydration.

Assessment of Status

The most sensitive marker of riboflavin status involves determining activity for the riboflavin-dependent enzyme, erythrocyte glutathione reductase, which reduces the antioxidant glutathione (Food and Nutrition Board 1998). An activity coefficient is determined by measuring activity of the enzyme with and without added FAD. When the erythrocyte glutathione reductase activity coefficient (EGRAC) is 1.2 to 1.4, riboflavin status is considered low; an EGRAC greater than 1.4 suggests riboflavin deficiency. Urinary riboflavin concentrations less than 40 μg/day also suggest poor status (Food and Nutrition Board 1998).

Dietary Intake and Status of Riboflavin in Active Adults

Most cross-sectional studies indicate that nonsupplementing female athletes meet dietary recommendations for riboflavin (Table 7.4) (Kaiserauer et al. 1989; Keith et al. 1989; Nieman et al. 1989; Worme et al. 1990; Faber and Benadé 1991; Keith and Alt 1991; Fogelholm et al. 1992; Rokitzki et al. 1994a; Beals and Manore 1998; Kopp-Woodroffe et al. 1999; Beshgetoor and Nichols 2003; Clark et al. 2003). However, some studies suggest that female athletes might be at risk for poor riboflavin status due to increased nutrient needs. Metabolic studies have examined the effect of exercise, dieting, or dieting plus exercise on riboflavin requirements in women (Belko et al. 1983, 1984, 1985; Winters et al. 1992); overall results suggest that riboflavin needs are higher in females engaging in exercise for fitness compared to sedentary controls. Belko et al. (1983, 1984, 1985) completed a series of metabolic studies examining the riboflavin status of active young women. In the first metabolic study (Belko et al. 1983), moderate physical activity (20 to 50 min/day, 6 days/week) increased riboflavin requirements to 1.4 mg/1000 kcal. Additional metabolic studies found that dieting (1200 to 1250 kcal/day) and dieting plus exercise increased

TABLE 7.4
Summary of Studies Examining Riboflavin Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Beals and Manore 1998	24 female athletes with subclinical eating disorders; 24 female control athletes	7-day weighed food record	Athletes with subclinical eating disorders: 2.2 ± 0.6 mg/day Control athletes: 2.7 ± 0.7 mg/day
Beshgetoor and Nichols 2003	25 female nonsupplementing master cyclists and runners	4-day food record	2.0 ± 0.5 mg/day
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 1.8 ± 0.7 mg/day Postseason: 1.2 ± 0.7 mg/day
Faber and Benadé 1991	30 field athletes (discus, hammer, and javelin throwers; shotputters) (10 female)	7-day food record	Female: 1.67 ± 0.42 mg/day
Fogelholm et al. 1992	17 Nordic skiers (8 female)	Four 7-day food records	Female: 2.4 ± 0.1 mg/day
Fogelholm et al. 1993	42 physically active college students (18 female)	Blood EGRAC	Female: 1.38
Kaiserauer et al. 1989	17 female distance runners (8 with amenorrhea; 9 with eumenorrhea)	3-day food record	Amenorrheic distance runners: 1.2 mg/day (mean) Eumenorrheic distance runners: 2.0 mg/day (mean)
Keith and Alt 1991	13 female athletes	3-day weighed food record; EGRAC; urinary riboflavin	Dietary 1.9 ± 0.9 mg/day EGRAC 1.06 ± 0.06 Urine 98 ± 51 μg/24 h

Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	1.6 ± 0.9 mg/day
Kopp-Woodroffe et al.1999	4 female amenorrheic runners	7-day weighed food record	1.6 ± 0.3 mg/day
Leydon and Wall 2002	19 jockeys (14 female)	7-day weighed food record	Female: 0.92 ± 0.34 mg/day
Nieman et al. 1989	347 marathon runners (56 female)	3-day food record	Female: 1.63 ± 0.67 mg/day
Rokitzki et al. 1994a	62 athletes (12 female handball athletes)	7-day weighed food record; EGRAC; whole blood riboflavin; urinary riboflavin	Dietary Female: 1.4 ± 1.7 mg/day EGRAC Female: 1.32 ± 0.62 Whole blood riboflavin Female: 353 ± 256 nmol/L Urine Female: 1.78 ± 1.84 μmol/L
Sato et al. 2011	19 college swimmers (13 female)	3-day food record; blood riboflavin	Dietary Female Preparation Period: 1.85 ± 1.06 mg/day Female Intensive-Training Period: 1.73 ± 0.44 mg/day Blood Female Preparation Period: 38 ± 10 ng/ml Female Intensive-Training Period: 93.1± 9.0 ng/ml
Worme et al. 1990	71 triathletes (21 female)	3-day food record	Female: 2.2 ± 0.9 mg/day

Source: Table adapted from Woolf, K., and Manore, M. M., 2006, B Vitamins and Exercise: Does Exercise Alter Requirement, *International Journal of Sport Nutrition and Exercise Metabolism* 16: 453–484. With permission.

[a] Values reported as mean ± standard deviation unless noted.

riboflavin requirements in active women (Belko et al. 1984, 1985). In these studies, 1.6 mg of riboflavin/1000 kcal (2 mg/day) was required to maintain good riboflavin status when the women were dieting for weight loss and exercising. In a similar study, active older women (2.5 h exercise/week) consuming a weight maintenance diet (1800 to 2000 kcal/day) required 1.8 mg/day of riboflavin to maintain good status (Winters et al. 1992). Thus, exercise, dieting, and dieting plus exercise increase the need for riboflavin above the 1998 RDA for women (Belko et al. 1983, 1984, 1985; Winters et al. 1992; Food and Nutrition Board 1998). The participants in these studies only performed moderate exercise (3 to 5 h/week) for fitness. Unfortunately, there are no metabolic studies available examining the status of riboflavin in female athletes who participate in strenuous exercise and competitive sports. However, if moderately active women have an increased need for riboflavin, then female athletes would have just as great a need, if not higher. Additionally, athletes who restrict dietary intake are at further risk of poor riboflavin status.

Riboflavin and Performance

Riboflavin supplementation does not enhance performance in male athletes who are already in good status but can be beneficial for those with poor status. For example, when Croatian adolescent boys (12 to 14 years) with poor nutrient status were provided with a riboflavin supplement for 5 weeks, riboflavin status improved significantly and physical fitness increased nonsignificantly (Subotičanec et al. 1990). Similarly, riboflavin supplementation would only be expected to enhance performance in female athletes in poor status.

NIACIN

Exercise-Related Functions

Niacin is found in the body in two coenzyme forms (nicotinamide adenine dinucleotide [NAD] and nicotinamide adenine dinucleotide phosphate [NADP]) (Table 7.1) (Wildman and Miller 2004; Gropper et al. 2009; Manore et al. 2009). Niacin serves in these coenzyme forms in oxidation-reduction reactions in metabolic pathways in the body. Niacin is needed during glycolysis, the conversion of pyruvate to acetyl CoA, and three dehydrogenase reactions of the TCA cycle. Niacin also functions in electron transfer in both fatty acid biosynthesis and β-oxidation. Niacin is required for the oxidative deamination of amino acids and the pentose phosphate pathway. These exercise-related functions of niacin are essential for optimal performance.

Food Sources and Recommended Intakes

Niacin is found in a variety of sources including brewer's yeast, fish, pork, beef, poultry, mushrooms, nuts, potatoes, whole grains, and enriched or fortified grains and cereals (Table 7.2). Niacin can also be endogenously synthesized from the essential amino acid tryptophan. For men and women ages 19 to 50 years, the EAR for niacin

(niacin equivalents) is 12 and 11 mg/day, respectively (Table 7.2). The RDA for niacin (niacin equivalents) for men and women ages 19 to 50 years is 16 and 14 mg/day, respectively (Food and Nutrition Board 1998). The UL for niacin is 35 mg/day and applies only to synthetic forms of niacin obtained from supplements or fortified foods (Food and Nutrition Board 1998). The UL was determined based on the flushing (i.e., burning, tingling, itching, and redness) that results after excess niacin intake.

Assessment of Status

The best markers of niacin status include urinary metabolites of the nutrient (Gibson 2005). Urinary excretion of <0.8 mg/day (<5.8 μmol/day) of N^1 methyl-nicotinamide indicates poor niacin status. Niacin status can also be assessed by the ratio of urinary N^1 methyl-2-pyridone-5-carboxamide (2-pyridone) to N^1 methyl-nicotinamide (NMN), with a ratio of less than one suggesting poor status (Shibata and Matsuo 1989).

Dietary Intake and Status of Niacin in Active Adults

Most female athletes, engaged in a variety of sports, report mean dietary intakes for niacin that meet dietary recommendations (Table 7.5) (Keith et al. 1989; Nieman et al. 1989; Worme et al. 1990; Faber and Benadé 1991; Beals and Manore 1998; Kopp-Woodroffe et al. 1999; Beshgetoor et al. 2003). However, a few studies have reported lower intakes by female athletes (Table 7.5). For example, female soccer players completed food records for 3 days on two occasions (pre- and postseason) (Clark et al. 2003). The mean dietary intakes for niacin during the postseason were much lower than the preseason (preseason 24.5 ± 8.5 mg/day; postseason 15.2 ± 6.3 mg/day). The athletes reported consuming significantly less energy during the postseason. In another study, female distance runners with amenorrhea reported a mean dietary intake for niacin less than the 1998 RDA but met the 1998 EAR (mean 12.2 mg/day) (Kaiserauer et al. 1989). Low intakes of niacin have also been reported by female jockeys with mean dietary intakes of niacin (8.29 ± 2.53 mg/day) less than both the 1998 RDA and EAR (Leydon and Wall 2002). Because these athletes restricted energy intake, dietary intakes of niacin were inadequate.

Only limited research has examined niacin status in athletes using a biochemical marker. Singh et al. (1993) examined dietary, blood, and urinary markers of niacin status in male and female ultramarathoners. Biochemical markers (blood, urine) of niacin status for these athletes were within the normal reference range. More research is needed that examines niacin status in the female athlete.

Niacin and Performance

Given niacin's metabolic roles, female athletes may consider supplementing with niacin in order to improve exercise performance; however, supplementation will likely provide no ergogenic benefit and may negatively impact performance. For example, 10 healthy participants (male and female) completed exercise tests on five occasions; nicotinic acid was provided during exercise as part of the study protocol

TABLE 7.5
Summary of Studies Examining Niacin Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Beals and Manore 1998	24 female athletes with subclinical eating disorders; 24 female control athletes	7-day weighed food record	Athletes with subclinical eating disorders: 25.1 ± 9.9 mg/day Control athletes: 29.4 ± 9.5 mg/day
Beshgetoor and Nichols 2003	25 female nonsupplementing master cyclists and runners	4-day food record	29.0 ± 9.0 mg/day
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 24.5 ± 8.5 mg/day Postseason: 15.2 ± 6.3 mg/day
Faber and Benadé 1991	30 field athletes (discus, hammer, and javelin throwers; shotputters) (10 female)	7-day food record	Female: 18.8 ± 3.8 mg/day
Kaiserauer et al. 1989	17 female distance runners (8 with amenorrhea; 9 with eumenorrhea)	3-day food record	Amenorrheic distance runners: 12.2 mg/day (mean) Eumenorrheic distance runners: 16.8 mg/day (mean)
Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	18.9 ± 9.9 mg/day
Kopp-Woodroffe et al. 1999	4 female amenorrheic runners	7-day weighed food record	20.7 ± 5.4 mg/day
Leydon and Wall 2002	19 jockeys (14 female)	7-day weighed food record	Female: 8.29 ± 2.53 mg/day
Nieman et al. 1989	347 marathon runners (56 female)	3-day food record	Female: 20.7 ± 9.7 mg/day
Worme et al. 1990	71 triathletes (21 female)	3-day food record	Female: 21.9 ± 6.4 mg/day

[a] Values reported as mean ± standard deviation unless noted.

(Murray et al. 1995). The nicotinic acid blunted the rise in free fatty acids normally associated with exercise and could potentially reduce the ability to perform.

VITAMIN B_6

Exercise-Related Functions

Vitamin B_6 plays an important role in the metabolic pathways required for exercise, principally amino acid metabolism and glycogen breakdown (Table 7.1) (Leklem 1990; Maughan 1999; Manore 1994, 2000; Lukaski 2004; Wildman and Miller 2004; Hansen and Manore 2005; Volpe 2007; Woolf and Manore 2006, 2007; Manore et al. 2000, 2009). Pyridoxal 5′-phosphate (PLP) is the active form of vitamin B_6 and performs the vitamin B_6 roles required for exercise in the human body. Vitamin B_6 serves as a coenzyme in transamination and deamination reactions, allowing the breakdown of amino acids, and in glycogen degradation, activating the rate-limiting enzyme glycogen phosphorylase. Vitamin B_6 is also needed for the endogenous synthesis of niacin.

Food Sources and Recommended Intakes

Food sources and recommended intakes of vitamin B_6 are summarized in Table 7.2. Good sources of vitamin B_6 include animal foods, such as meat, fish, and poultry, noncitrus fruits and juices, whole-grain products, and fortified cereals (Food and Nutrition Board 1998). The EAR and RDA for vitamin B_6 are 1.1 and 1.3 mg/day, respectively, for men and women ages 19 to 50 years (Food and Nutrition Board 1998). Because vitamin B_6 plays a major role in protein metabolism, recommendations have historically been based upon the amount of protein ingested. Hansen and Manore (2005) suggest athletes may require two to three times the RDA of vitamin B_6 due to their increased activity levels and protein requirements. The UL for vitamin B_6 is 100 mg/day.

Assessment of Status

Several measures are commonly used to assess vitamin B_6 status (Food and Nutrition Board 1998). The best direct or static marker of vitamin B_6 status is plasma PLP concentrations, a good indicator of tissue stores. Plasma PLP should be ≥20 nmol (≥4.94 ng/ml) in order to be in good status (Lui et al. 1985; Food and Nutrition Board 1998). Another static measure of vitamin B_6 is urinary 4-pyridoxic acid; >3 μmol/day has been suggested to indicate adequate status (Leklem 1990).

Functional markers of vitamin B_6 status include the erythrocyte alanine transaminase activity coefficient (EALTAC) and erythrocyte aspartate transaminase activity coefficient (EASTAC). These enzymes, erythrocyte alanine transaminase and erythrocyte aspartate transaminase, require PLP as a coenzyme. High activity of either coefficient (>1.25 for EALTAC and >1.8 for EASTAC) suggests poor long-term status of vitamin B_6 (Leklem 1990; Food and Nutrition Board 1998; Gibson 2005).

Tryptophan loading can also be used as a functional measure of vitamin B_6 status (Gropper et al. 2009). In the body, the amino acid tryptophan can be converted to

niacin using vitamin B_6 as a coenzyme. If vitamin B_6 status is poor, a side reaction produces the metabolite xanthurenic acid, which is excreted in the urine. Poor status of vitamin B_6 is noted when the urinary excretion of xanthurenic acid exceeds 25 mg in 6 hours (Gropper et al. 2009).

Because homocysteine metabolism is influenced by vitamin B_6, folate, and B_{12} status, homocysteine concentrations can be used as a functional marker of B_6 status. The cutoff value for elevated plasma homocysteine is >12 μmol/L (1.62 mg/L) (Rasmussen et al. 1996). Higher values, such as >14 μmol/L (1.89 mg/L) and >16 μmol/L (2.16 mg/L) have also been suggested (Selhub et al. 1993; Food and Nutrition Board 1998).

Dietary Intake and Status of Vitamin B_6 in Active Adults

Most studies document adequate mean vitamin B_6 intakes among female athletes when compared to the 1998 RDA (1.3 mg/day) and EAR (1.1 mg/day) (Table 7.6). However, studies comparing mean dietary intakes to the 1980 RDA (2 mg/day for adult females) or 1989 RDA (1.6 mg/day for adult females) would identify more female athletes with inadequate dietary intakes for vitamin B_6. For example, Keith et al. (1989) examined the micronutrient intakes of eight female cyclists using 3-day weighed food records and compared them to the 1980 RDA. The average intake of vitamin B_6 was 1.8 ± 1.1 mg/day or 90% of the 1980 RDA (2 mg/day). Three, greater than one-third, of the participants had intakes less than two-thirds of the 1980 RDA for vitamin B_6. Using more recent recommendations, the average intake of vitamin B_6 in this study met the 1998 RDA (1.3 mg/day) and those with marginal intakes met the 1998 EAR (1.1 mg/day). In another study, Faber and Benadé (1991) examined the dietary intake of 10 female field athletes and the results were compared to the 1989 RDA (2 mg/day for men and 1.6 mg/day for women) for vitamin B_6. Sixty percent of the female field athletes consumed less than 100% of the RDA for vitamin B_6 (2.8 ± 1.1 mg/day for males and 1.6 ± 0.4 mg/day for females). Ten percent of the female field athletes had a vitamin B_6 intake less than 67%, classified as inadequate. In another study, dietary intakes from female collegiate heavyweight rowers (n = 16) were examined (Steen et al. 1995). Only 70% of the athletes met 100% of the 1989 RDA for vitamin B_6. The authors attribute the decreased intake of vitamin B_6 to the low intake of dairy, beef, and poultry. Although the participants were heavyweight rowers, those who did not meet the RDA may have been concerned about making weight and thus restricted their energy intake.

Other studies reported adequate intakes of vitamin B_6 by female athletes. Using the 1998 EAR, adequate dietary intakes for vitamin B_6 have been reported for female athletes with subclinical eating disorders (Beals and Manore 1998), master athletes (Beshgetoor and Nichols 2003), field athletes (Faber and Benadé 1991), recreational athletes (Joubert and Manore 2008), distance runners (Kaiserauer et al. 1989; Manore et al. 1989; Nieman et al. 1989), cyclists (Keith and Alt 1991), runners with amenorrhea (Kopp-Woodroffe et al. 1999), handball athletes (Rokitzki et al. 1994c), and triathletes (Worme et al. 1990). Historically, results are mixed in regards to vitamin B_6 intake among female athletes due to revised dietary recommendations. However, the RDA for vitamin B_6 has decreased with the latest guidelines, and most intakes from previous studies would meet the current EAR of 1.1 mg/day.

In spite of reported intakes that meet dietary recommendations, several studies have documented poor vitamin B_6 status (Telford et al. 1992; Fogelholm et al. 1993; Manore 1994, 2000; Rokitzki et al. 1994b). For example, Telford et al. (1992) studied 86 male and female athletes before and after an 8-month training period. Half of the subjects ($n = 42$) consumed a multivitamin/mineral supplement and half took a placebo ($n = 44$). Although dietary intakes met recommendations at baseline, 59% of the athletes had poor vitamin B_6 status while consuming their typical diets. Eight months later, 41% of the athletes on the placebo and 10% of the athletes on the supplement had poor vitamin B_6 status, respectively. Raczynski and Szczepanska (1993) examined vitamin B_6 status in 1753 elite male and female Polish athletes from the years 1987 to 1992. Dietary intakes for vitamin B_6 were not reported in this study. Using EASTAC, the risk of poor status averaged 6% in all athletes over the 6-year period (range 2 to 16%). Endurance athletes had the highest prevalence of poor vitamin B_6 status (13%) while those athletes engaging in team sports had a 10% prevalence rate of poor vitamin B_6 status. The risk of poor vitamin B_6 status was highest in pre-Olympic years (16%) and lowest in Olympic years (3%).

Exercise also appears to influence metabolism of vitamin B_6. Exercise increases blood concentrations of PLP, the active form of B_6 in the blood (Manore et al. 1987; Leklem 1990; Hoffman et al. 1991; Crozier et al. 1994). The PLP may be converted to 4-pyridoxic acid and then lost in the urine (Leklem and Shultz 1983; Leklem 1990; Hoffman et al. 1991; Crozier et al. 1994). Thus, exercise may increase the turnover and loss of vitamin B_6 from the body (Manore et al. 1987; Crozier et al. 1994). Research has documented higher 4-pyridoxic acid losses in active individuals compared to sedentary controls or periods of inactivity (Manore et al. 1989). Higher 4-pyridoxic acid losses have also been documented after strenuous physical activity in active men and women (Crozier et al. 1994).

Vitamin B_6 and Performance

Because vitamin B_6 plays such an important role in metabolic processes important for activity, researchers have examined whether supplemental vitamin B_6 enhances exercise performance. For instance, male and female swimmers were provided with a high dose of vitamin B_6 for 6 months (Lawrence et al. 1975). No improvements were noted in exercise performance after the supplementation. Additionally, Manore and Leklem (1988) found that 7 weeks of vitamin B_6 supplementation actually lowered circulating free fatty acid concentrations during exercise in women. Thus, female athletes should focus on achieving good vitamin B_6 status through a nutrient-dense diet. High-dose supplementation is not recommended as a mechanism to enhance performance.

PANTOTHENIC ACID

Exercise-Related Functions

Pantothenic acid functions as a component of two compounds (coenzyme A [CoA] and acyl carrier protein) involved in energy metabolism (Table 7.1) (Food and

TABLE 7.6
Summary of Studies Examining Vitamin B_6 Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Beals and Manore 1998	24 female athletes with subclinical eating disorders; 24 female control athletes	7-day weighed food record	Athletes with subclinical eating disorders: 2.1 ± 0.8 mg/day Control athletes: 2.5 ± 0.8 mg/day
Beshgetoor and Nichols 2003	25 female nonsupplementing master cyclists and runners	4-day food record	3 ± 1 mg/day
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 1.8 ± 0.6 mg/day Postseason: 1.1 ± 0.6 mg/day
Faber and Benadé 1991	30 field athletes (discus, hammer, and javelin throwers; shotputters) (10 female)	7-day food record	Female: 1.62 ± 0.42 mg/day
Fogelholm et al. 1993	42 physically active college students (18 female)	Blood EASTAC	Female: 1.92 (mean)
Joubert and Manore 2008	64 recreational athletes (38 female)	7-day weighed food record; plasma PLP; plasma homocysteine	Female low physical activity: Vitamin B_6: 2.2 ± 1.6 mg/day Plasma PLP: 48.1 ± 18.6 nmol/L Plasma homocysteine: 7.4 ± 1.6 µmol/L Female high physical activity: Vitamin B_6: 2.4 ± 0.7 mg/day Plasma PLP: 49.6 ± 19.7 nmol/L Plasma homocysteine: 7.4 ± 1.1 µmol/L

Kaiserauer et al. 1989	17 female distance runners (8 with amenorrhea; 9 with eumenorrhea)	3-day food record	Amenorrheic distance runners: 1.5 mg/day (mean) Eumenorrheic distance runners: 2.1 mg/day (mean)
Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	1.8 ± 1.1 mg/day
Kopp-Woodroffe et al.1999	4 female amenorrheic runners	7-day weighed food record	1.7 ± 0.5 mg/day
Leydon and Wall 2002	19 jockeys (14 female)	7-day weighed food record	Female: 0.90 ± 0.49 mg/day
Manore et al. 1989	10 female long-distance runners	3-day food record	1.8 ± 0.9 mg/day
Nieman et al. 1989	347 marathon runners (56 female)	3-day food record	Female: 1.64 ± 0.75 mg/day
Rokitzki et al. 1994a	57 athletes (12 female handball athletes)	7-day weighed food record; EASTAC; whole blood vitamin B_6; urinary 4-pyridoxic acid	Dietary Female: 1.36 ± 0.97 mg/day EGOT Female: 1.43 (geometric mean) Whole-blood vitamin B_6 Female: 0.037 nmol/L (geometric mean) Urine pyridoxic acid Female: 3.22 µmol/g Cr (geometric mean)
Worme et al. 1990	71 triathletes (21 female)	3-day food record	Female: 2.0 ± 0.9 mg/day

Source: Adapted from Woolf, K., and Manore, M. M., 2006, B Vitamins and Exercise: Does Exercise Alter Requirement, *International Journal of Sport Nutrition and Exercise Metabolism* 16: 453–484. With permission.

[a] Values reported as mean ± standard deviation unless noted.

Nutrition Board 1998; Wildman and Miller 2004; Gropper et al. 2009; Manore et al. 2009). Coenzyme A activates intermediates in the body, allowing them to be metabolized (i.e., acetyl CoA, succinyl CoA, propionyl CoA, malonyl CoA). Acyl carrier protein is required in an early step in fatty acid biosynthesis as part of the large enzyme fatty acid synthase. These two compounds are necessary for the metabolism of fuel in the body.

Food Sources and Recommended Intakes

Table 7.2 summarizes food sources and recommended dietary intakes for pantothenic acid. Pantothenic acid is available in a wide variety of foods including liver, meat, fish, poultry, milk, and eggs (Food and Nutrition Board 1998). Whole-grain products, oat cereals, yeast, and legumes are also good sources. The Adequate Intake (AI) for pantothenic acid for adults 19 years or older is 5 mg/day (Food and Nutrition Board 1998). An AI is established for a nutrient when insufficient data are available to determine an EAR and RDA. Since adverse effects of panthothenic acid have not been associated with high intakes, no UL has been determined for this nutrient.

Assessment of Status

Status of pantothenic acid is commonly assessed using blood and urine concentrations of the nutrient (Cohenour and Calloway 1972; Sauberlich 1999). Normal concentrations for pantothenic acid in whole blood have been reported to be 1.57 to 2.66 μmol/L (0.344 to 0.583 ng/ml) (Wittwer et al. 1989). Blood concentrations of pantothenic acid do not always reflect changes in dietary intake of pantothenic acid. Urinary pantothenic acid is considered to be a better status marker; excretion of <1 mg/day suggests poor status. Urinary excretion of pantothenic acid on a typical American diet is approximately 2.6 mg/day (Tarr et al. 1981).

Dietary Intake and Status of Pantothenic Acid in Active Adults

Unfortunately, only limited research has examined pantothenic acid status in athletes, and these studies have only included dietary assessments (Table 7.7). The existing research suggests that athletes with low energy intakes may have inadequate pantothenic acid. For example, Clark et al. (2003) examined dietary intakes of pantotheic acid in female soccer players twice during the season. Both mean dietary intakes preseason and postseason did not meet the AI for pantothenic acid (preseason 3.1 ± 2.2 mg/day; postseason 2.0 ± 1.3 mg/day). In another study, female distance runners (amenorrheic and eumenorrheic) completed 3-day food records that were analyzed for intake of pantothenic acid (Kopp-Woodroffe et al. 1999). Athletes with amenorrhea did not meet the AI for pantothenic acid (mean 3.6 mg/day) while the athletes with eumenorrhea did (mean 5.1 mg/day). Furthermore, female cyclists also reported mean dietary intakes low in pantothenic acid (3.5 ± 2.2 mg/day) (Keith et al. 1989). More research is needed that examines pantothenic acid intake and status in female athletes.

TABLE 7.7
Summary of Studies Examining Pantothenic Acid Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 3.1 ± 2.2 mg/day Postseason: 2.0 ± 1.3 mg/day
Kaiserauer et al. 1989	17 female distance runners (8 with amenorrhea; 9 with eumenorrhea)	3-day food record	Amenorrheic distance runners: 3.6 mg/day (mean) Eumenorrheic distance runners: 5.1 mg/day (mean)
Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	3.5 ± 2.2 mg/day

[a] Values reported as mean ± standard deviation unless noted.

Pantothenic Acid and Performance

Due to the role of pantothenic acid in energy metabolism, research has examined whether supplementation of this nutrient enhances performance. One study examined the impact of pantothenic acid as an ergogenic aid in six highly trained male and female cyclists (Webster 1998). The participants were randomly assigned to a supplement with thiamin and pantothenic acid or a placebo. After completing a 50 km ride, no differences were found between the treatments in measures of exercise metabolism or performance; thus, pantothenic acid supplementation did not impact exercise performance.

BIOTIN

Exercise-Related Functions

The B-vitamin biotin serves as a coenzyme for four carboxylase reactions integral to the metabolism of carbohydrates, fatty acids, and amino acids and essential for physical activity (Table 7.1) (Wildman and Miller 2004; Gropper et al. 2009; Manore et al. 2009). Biotin catalyzes pyruvate carboxylase, a mitochondrial enzyme that converts pyruvate to form oxaloacetate. The oxaloacetate can be used in the TCA cycle or for gluconeogenesis. Biotin catalyzes acetyl CoA carboxylase, the rate-limiting enzyme in the initiation of fatty acid synthesis. Propionyl CoA carboxylase, another biotin-dependent enzyme, metabolizes odd-chain-length fatty acids and the amino acids isoleucine, threonine, and methionine. Another biotin-dependent enzyme (β-methylcrotonyl CoA carboxylase) is involved in the catabolism of the amino acid leucine.

Food Sources and Recommended Intakes

Table 7.2 summarizes food sources and recommended intakes of biotin. Although biotin is widely available in many foods, the best dietary sources of biotin are liver, whole-grain cereals, wheat bran, brewer's yeast, nuts, and legumes (Food and Nutrition Board 1998). Biotin is also produced by bacteria in the colon. Raw egg whites contain the protein avidin, which has been found to prevent biotin absorption. The AI for biotin for adults 19 years and older is 30 µg/day (Food and Nutrition Board 1998). Because toxicity symptoms for biotin have not been reported even at high dietary intakes, a UL for biotin has not been defined.

Assessment of Status

Status of biotin can be assessed using blood and urine concentrations. However, plasma concentrations do not always correlate with dietary intake or nutrient status (Food and Nutrition Board 1998). Urinary excretion of biotin (poor status <6 µg/day) and biotin metabolites are more sensitive indicators of status (Gropper et al. 2009).

Dietary Intake and Status of Biotin in Active Adults

Although biotin is an essential nutrition for health and performance, only limited research has examined biotin intakes and status in female athletes (Table 7.8). In one study, female soccer players completed food records for 3 days twice during the season (Clark et al. 2003). Both mean dietary intakes pre- and postseason did not meet the AI for biotin (preseason 11.3 ± 9.8 µg/day; postseason 13.3 ± 26.5 µg/day). Biotin status (urine and blood) has also been measured in male and female ultramarathoners (Singh et al. 1993). Although the mean blood biotin concentration for the athletes was within the defined reference range, the urinary excretion of biotin varied widely among the athletes.

Biotin and Performance

The impact of biotin on exercise performance has yet to be determined in the research literature. Further work needs to be completed.

TABLE 7.8
Summary of Studies Examining Biotin Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 11.3 ± 9.8 µg/day Postseason: 13.3 ± 26.5 µg/day

[a] Values reported as mean ± standard deviation unless noted.

CHOLINE

Exercise-Related Functions

Choline functions in the body as a precursor for acetylcholine, phospholipids, and the methyl donor betaine (Table 7.1) (Food and Nutrition Board 1998; Penry and Manore 2008). The body requires choline for the synthesis and release of acetylcholine, the neurotransmitter at neuromuscular junctions (Penry and Manore 2008). Choline is the dietary component of phosphatidylcholine (lecithin), the main phospholipid in cell membranes. In the body, choline can be converted to betaine, which is involved in methyl-group metabolism. These functions of choline are essential for overall health and are important for exercise.

Food Sources and Recommended Intakes

Choline is found in a variety of foods including milk, liver, eggs, soybeans, legumes, nuts, seeds, and wheat germ (Table 7.2). Choline can also be synthesized in the body. The Institute of Medicine made dietary recommendations for choline for the first time in 1998 (Food and Nutrition Board 1998; Ziesel 2000). The AI for choline for adults is 550 mg/day for men and 425 mg/day for women (Table 7.2). Although an AI for choline has been established, the choline requirement may be met by endogenous synthesis during some stages of the life cycle (Food and Nutrition Board 1998; Ziesel 2000). The UL for choline is 3500 mg/day. Symptoms of choline toxicity include hypotension, sweating, diarrhea, and a fishy body odor (Food and Nutrition Board 1998).

Assessment of Status

Both plasma choline and phosphatidylcholine are good markers of choline status (Food and Nutrition Board 1998). Plasma choline concentrations vary from 7 to 20 μmol/L, with typical concentrations around 10 μmol/L (Food and Nutrition Board 1998). Unfortunately, plasma choline concentrations do not typically drop until severe deficiency is present. Normal plasma phosphatidylcholine concentrations range from 1.0 to 1.5 mmol/L (Ziesel et al. 1980, 1991). Erythrocyte and urinary choline concentrations have also been used as status markers for choline (Gropper et al. 2009).

Dietary Intake and Status of Choline in Active Adults

Dietary intakes of choline have not been reported in the research literature because the choline content of foods is not widely available. However, studies have examined acute changes in plasma choline concentrations with exercise (Table 7.9). For example, 23 male and female runners participated in a study before and after a marathon (Buchman et al. 1999). Markers of choline status in blood and urine were collected from the research participants 14 days before the race, immediately after the race, and 2 days later. In this study, the participants exhibited a decrease in both free and phospholipid-bound choline concentrations after the marathon. As food composition data become available for choline, more research is needed on the dietary intakes of female athletes.

TABLE 7.9
Summary of Studies Examining Choline Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Buchman et al. 2000	12 marathon runners (7 male; 5 female)	Plasma choline; phospholipid bound choline; urinary choline	Plasma choline 8.0 ± 1.2 μmol/L Plasma phospholipid choline 2135 ± 522 μmol/L Urine 13.2 ± 6.8 μmol/5 h
Buchman et al. 1999	23 marathon runners (male and female)	Plasma choline; phospholipid bound choline; urinary choline	Plasma choline 19.2 ± 4.5 μmol/L Phospholipid-bound choline 2565.2 ± 516.4 μmol/L Urine 15.5 ± 8.8 μmol/5 h
Von Allwörden et al. 1993	10 triathletes (6 male; 4 female)	Plasma choline	Group A: 12.08 ± 1.71 μmol/L Group B: 10.76 ± 0.73 μmol/L Group C: 11.00 ± 0.71 μmol/L

[a] Values reported as mean ± standard deviation unless noted.

Choline and Performance

Research has examined the impact of choline supplementation on athletic performance. In a randomized study, researchers examined if oral lecithin supplementation given prior to the day of a marathon would maintain plasma free and urinary choline concentrations, as well as improve performance (Buchman et al. 2000). Twelve male and female marathon runners were randomly assigned to receive lecithin supplementation or a placebo; all subjects completed the marathon. While the short-term lecithin supplementation maintained normal choline concentrations during the marathon, it did not enhance performance.

Similarly, male and female triathletes (n = 10) consumed either a placebo or lecithin supplement (0.2 g/kg body weight) during three different exercise protocols (Von Allwörden et al. 1993). The triathletes who ingested the placebo saw a significant decrease in plasma choline concentrations, while those consuming the lecithin supplement maintained choline status. More research is needed examining the impact of choline supplementation and exercise performance in female athletes.

FOLATE

Exercise-Related Functions

Folate performs many functions critical for active individuals, including the formation of red blood cells, tissue repair and maintenance, and DNA synthesis required for the production of new cells (Table 7.1) (Food and Nutrition Board 1998; Maughan 1999; Manore 1999, 2000; Lukaski 2004; Wildman and Miller 2004; Woolf and

Manore 2006, 2007; Volpe 2007; Manore et al. 2000, 2009; Gropper et al. 2009). In the body, the active form of folate is tetrahydrofolate (THF), which functions as a coenzyme to accept single-carbon groups from other compounds generated during the metabolism of amino and nucleic acids (Pancharuniti et al. 1994; Savage and Lindenbaum 1995; Wildman and Miller 2004). THF is essential for cells undergoing cell division, especially those with short life spans (i.e., enterocytes, red blood cells). THF functions as a coenzyme in DNA synthesis (the methylation of deoxyuridylic acid to thymidylic acid), purine synthesis, red blood cell production (the conversion of megaloblasts into mature red blood cells), and the metabolism of some amino acids (histidine to glutamic acid, glycine to serine, and homocysteine to methionine) (Food and Nutrition Board 1998; Gropper et al. 2009). Because of folate's roles in red blood cell production and tissue repair and maintenance, folate is an important nutrient for active women.

Food Sources and Recommended Intakes

Food sources and recommended intakes for folate are summarized in Table 7.2. Folate is found in animal products, particularly organ meats, leafy green vegetables, fortified cereals and grains, nuts, legumes, citrus fruits and juices, and brewer's yeast (Food and Nutrition Board 1998). In the United States, mandatory fortification of refined grain products began in 1998 (Food and Nutrition Board 1998). Fortification was designed to provide at least 100 μg/day of folic acid to the daily intake for the average person and ensure that 50% of women of childbearing age receive 400 μg/day of folic acid. The EAR for folate is 320 μg/day for men and women 19 years or older (Food and Nutrition Board 1998). The 1998 RDA for folate is 400 μg/day of Dietary Folate Equivalents (DFEs) for men and women 19 years and older (Food and Nutrition Board 1998). The DFEs adjust for the lower bioavailability of natural food sources of folate compared to synthetic folic acid (Gregory 1997; Food and Nutrition Board 1998). Because folate is important for the synthesis of red blood cells, DNA production, and protein metabolism, athletes may require a higher intake of folate. The UL for folate is 1000 μg/day and applies only to synthetic forms obtained from supplements and fortified foods. Supplementation with folic acid above the UL may exacerbate the neuropathy seen in vitamin B_{12}–deficient individuals (Food and Nutrition Board 1998).

Assessment of Status

Folate is most commonly assessed by directly measuring serum or plasma concentrations, reflecting recent dietary intake. A serum folate concentration of <3 ng/ml (7 nmol/L) suggests poor status (Food and Nutrition Board 1998). Red blood cell concentrations reflect long-term status, but this measurement is more indicative of tissue folate status at the time the red blood cells were synthesized (and not changes in recent dietary folate intake). A red blood cell folate concentration of <140 ng/ml (305 nmol/L) suggests poor folate status but may also reflect poor vitamin B_{12} status (Food and Nutrition Board 1998).

Plasma homocysteine concentrations can be used as a functional marker of folate status. Because folate is required to convert homocysteine to methionine, a deficiency

in folate can increase plasma homocysteine concentrations (Mason and Miller 1992; Gropper et al. 2009). Folate has been suggested to have a greater potential for decreasing plasma homocysteine concentrations than vitamin B_{12} or vitamin B_6. The cutoff value for elevated plasma homocysteine is >12 μmol/L (1.62 mg/L) (Rasmussen et al. 1996). Other values, such as >14 μmol/L (1.89 mg/L) and >16 μmol/L (2.16 mg/L), have also been used (Selhub et al. 1993; Food and Nutrition Board 1998).

Folate status follows a four-stage model. Stage I (negative folate balance) is characterized by a depletion of folate stores in the body (Herbert and Das 1994). Stage II (folate depletion) is characterized by low serum folate concentrations and decreased red blood cell folate concentrations. Stage III (folate-deficient erythropoiesis) is characterized by a combination of stage II symptoms and impairment of DNA synthesis. During stage III, red blood cell synthesis is impaired and the signs of anemia develop (Gibson 2005). Stage IV (folate-deficiency anemia) is characterized by anemia and an elevated mean corpuscular volume (MCV) (Herbert and Das 1994).

Dietary Intake and Status of Folate in Active Adults

Using the 1998 EAR (Food and Nutrition Board 1998) for folate (320 μg/day DFE) as the criteria, multiple studies have reported inadequate mean dietary intakes in female athletes (Kaiserauer et al. 1989; Keith et al. 1989; Manore et al. 1989; Nieman et al. 1989; Worme et al. 1990; Faber and Benadé 1991; Spodaryk et al. 1996; Beals and Manore 1998; Kopp-Woodroffe et al. 1999; Leydon and Wall 2002; Clark et al. 2003) (Table 7.10). Reported mean dietary intakes for folate in female athletes range from 132 to 428 μg/day (Kaiserauer et al. 1989; Keith et al. 1989; Manore et al. 1989; Nieman et al. 1989; Worme et al. 1990; Faber and Benadé 1991; Spodaryk et al. 1996; Beals and Manore 1998; Kopp-Woodroffe et al. 1999; Leydon and Wall 2002; Beshgetoor and Nichols 2003; Clark et al. 2003; Joubert and Manore 2008). For instance, Keith et al. (1989) examined the micronutrient intakes of eight female cyclists using 3-day food records. The average intake of folate was 303 ± 305 μg/day, less than the 1998 EAR (320 μg/day). Similarly, Clark and colleagues examined the pre- and postseason dietary intakes for folate of 13 intercollegiate women soccer players (preseason 271 ± 130 μg/day; postseason 186 ± 113 μg/day) (Clark et al. 2003). No participant was found to have marginal intake pre- or postseason for folate when compared to the 1989 RDA; however, both pre- and postseason mean intakes failed to meet the 1998 RDA or EAR (320 μg/day).

In contrast to the above data, a few studies have found higher dietary intakes for folate among female athletes. Beshgetoor and Nichols (2003) examined the folate intake of 25 female master athletes (cyclists and runners). The mean intake from food for athletes not using dietary supplements was 402 ± 115 μg/day (101% of the RDA) and was higher than the mean intake from food for athletes using dietary supplements. Recently, Joubert and Manore (2008) reported mean dietary intakes in female recreational athletes above the 1998 RDA. Higher intakes in the recent literature may be a reflection of folic acid fortification.

Unfortunately, research examining folate status among female athletes is limited. Matter et al. (1987) examined folate status in 85 nonsupplementing female marathon runners and found that 33% (n = 28) had poor folate status. However, no assessment

of dietary folate intake was completed in this study. When Beals and Manore (1998) examined folate status in female athletes (~50% reported supplementing), 4% of their athletes were in poor folate status (plasma folate ≤1.8 nmol/L or ≤3 ng/ml). Other studies have found good folate status in female recreational athletes (Joubert and Manore 2008; Di Santolo et al. 2009), runners (both amenorrheic and eumenorrheic) (Hoch et al. 2010), and endurance athletes (Herrmann et al. 2005).

More recent studies have used plasma homocysteine as a functional marker of folate status and an indicator of cardiovascular health. Borrione et al. (2007) examined folate status in winter elite male and female athletes and reported an excess prevalence of hyperhomocysteinemia. Plasma homocysteine concentrations were higher during the training versus the recovery period. During the recovery period, the female athletes had significantly lower plasma homocysteine and higher plasma folate concentrations than the male athletes. For both genders, folate concentrations were negatively correlated with homocysteine concentrations. Joubert and Manore (2008) examined plasma homocysteine concentrations in active and less active women. When controlling for plasma B-vitamin concentrations, the plasma homocysteine concentrations were higher in the active women. Recently, Papapanagiotou et al. (2011) reported an increase in serum homocysteine concentrations following a football and hockey match. Dehydration and decreased blood volume after strenuous exercise may also be a factor in the results seen in these studies. However, athletes should increase intake of nutrients associated with homocysteine metabolism in order to improve nutritional status and decrease cardiovascular risk.

These studies suggest that many active women are at risk for poor folate status due to inadequate dietary intakes. Because of the role of folate in the prevention of neural tube defects, adequate folate is critical for female athletes in their childbearing years. Thus, it is imperative that female athletes increase their daily intake of folate to the current RDA of 400 μg/day.

Folate and Performance

Unfortunately, research examining folate and exercise performance is quite limited. In one study, female marathon runners with poor folate status were given a high-dose folate supplement (5 mg/day) for 11 weeks (Matter et al. 1987). Although serum folate concentrations improved during the study, no differences were seen in exercise performance or metabolic markers (i.e., peak blood lactate concentrations, running speed at blood lactate turn point) after supplementation. In another study, researchers also found no beneficial effect of a vitamin and mineral supplement (containing folate) on exercise performance in trained athletes (Weight et al. 1988). Unfortunately, information was not provided on the nutrient status of the athletes at study entry.

VITAMIN B_{12}

Exercise-Related Functions

Like folate, vitamin B_{12} plays important roles for active women, such as DNA synthesis and cellular development, red blood cell formation, fatty acid metabolism, and

TABLE 7.10
Summary of Studies Examining Folate Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Beals and Manore 1998	24 female athletes with subclinical eating disorders; 24 female control athletes	7-day weighed food record; serum folate	Dietary Athletes with subclinical eating disorders: 306 ± 157 µg/day Control athletes: 364 ± 99 µg/day Blood Athletes with subclinical eating disorders: 20.2 ± 7.3 nmol/L Control athletes: 19.3 ± 8.4 nmol/L
Beshgetoor and Nichols 2003	25 female nonsupplementing master cyclists and runners	4-day food record	402 ± 115 µg/day
Borrione et al. 2007	103 winter elite athletes (44 females)	Plasma folate; plasma homocysteine	Plasma folate, female: 19.1 ± 5.6 nmol/L Plasma homocysteine, female: 10.7 ± 5.9 µmol/L
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 271 ± 130 µg/day Postseason: 186 ± 113 µg/day
Di Santolo et al. 2009	124 female recreational athletes	Plasma folate; plasma homocysteine	Plasma folate: 5.10 ± 5.50 ng/ml (median) Plasma homocysteine: 9.14 µmol/L (median)
Faber and Benadé 1991	30 field athletes (discus, hammer, and javelin throwers; shotputters) (10 female)	7-day food record	Female: 230 ± 64 µg/day
Hoch et al. 2010	Healthy female runners: (10 amenorrheic, 10 eumenorrheic)	Serum folate	Amenorrheic: 20.8 ± 4.6 ng/ml Eumenorrheic: 17.4 ± 5.4 ng/ml
Joubert and Manore 2008	64 recreational athletes (38 female)	7-day weighed food records; plasma folate; plasma homocysteine	Female low physical activity: Folate: 428 ± 125 µg/day Plasma folate: 32.63 ± 8.99 nmol/L Plasma homocysteine: 7.4 ± 1.6 µmol/L Female high physical activity: Folate: 511 ± 105 µg/day Plasma folate: 35.20 ± 6.83 nmol/L Plasma homocysteine: 7.4 ± 1.1 µmol/L

Kaiserauer et al. 1989	17 female distance runners (8 amenorrheic; 9 eumenorrheic)	3-day food record	Amenorrheic distance runners: 198.5 µg/day (mean) Eumenorrheic distance runners: 276.4 µg/day (mean)
Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	303 ± 305 µg/day
Kopp-Woodroffe et al. 1999	4 female amenorrheic runners	7-day weighed food record; plasma folate	Folate: 250 ± 105 µg/day Plasma folate: 4.2 ± 1.7 mg/ml
Leydon and Wall 2002	19 jockeys (14 female)	7-day weighed food record	Female: 132 ± 52 µg/day
Manore et al. 1989	10 female long-distance runners	3-day food record	252.7 ± 111.4 µg/day
Nieman et al. 1989	347 marathon runners (56 female)	3-day food record	Female: 266 ± 150 µg/day
Spodaryk et al. 1996	40 trained female athletes	7-day food record	190 ± 33 µg/day
Telford et al. 1992	86 athletes (36 female)	Blood folate	Female: 14.2 ± 1.8 nmol/L
Weight et al. 1988	30 well-trained male runners	5-day food record; serum folate; RBC folate	Dietary 264.6 ± 100.1 µg/day Serum folate 11.1 ± 4.3 nmol/L RBC folate 630 ± 148 nmol/L
Worme et al. 1990	71 triathletes (21 female)	3-day food record	Female: 302 ± 1123 µg/day

Source: Adapted from Woolf, K., and Manore, M. M., 2006, B Vitamins and Exercise: Does Exercise Alter Requirement, *International Journal of Sport Nutrition and Exercise Metabolism* 16: 453–484. With permission.

[a] Values reported as mean ± standard deviation unless noted.

tissue repair and maintenance (especially in the nervous system) (Table 7.1) (Maughan 1999; Manore 1999, 2000; Lukaski 2004; Wildman and Miller 2004; Volpe 2007; Woolf and Manore 2006, 2007; Manore et al. 2000, 2009). Vitamin B_{12} functions as a coenzyme for two enzymes, methionine synthase and methylmalonyl-CoA mutase (Food and Nutrition Board 1998). Methionine synthase requires vitamin B_{12} to transfer a methyl group to homocysteine to form methionine and tetrahydrofolate. During this reaction, folate is converted from an inactive (methyltetrahydrofolate) to an active form (tetrahydrofolate) of the vitamin. Without sufficient vitamin B_{12}, folate remains "trapped" in the inactive form. Thus, the coenzyme activities of folate cannot be performed (i.e., DNA synthesis, purine synthesis, red blood cell formation, amino acid interconversions) and a folate deficiency develops. Methylmalonyl CoA mutase requires vitamin B_{12} to convert methylmalonyl CoA to succinyl CoA, a step in the metabolism of odd-chain fatty acids for energy production. Vitamin B_{12} also plays a role in the breakdown of certain amino acids and in maintaining neural tissue.

Food Sources and Recommended Intakes

Vitamin B_{12} is found only in foods from animal origin (Table 7.2). The best sources include meat, fish, poultry, egg, and milk and milk products, such as cheese and yogurt (Food and Nutrition Board 1998). As a result, vegetarian athletes may have poor status of vitamin B_{12} and need to consume B_{12}-fortified foods or a B_{12} supplement in order to meet their needs (Barr and Rideout 2004). Fortunately, many vegetarian food products are fortified with vitamin B_{12}. The EAR for vitamin B_{12} is 2.0 μg/day for men and women ages 19 to 50 years (Food and Nutrition Board 1998) (Table 7.2). In 1998, the Food and Nutrition Board increased the RDA to 2.4 μg/day for men and women ages 19 to 50 years (Food and Nutrition Board 1998). There is no UL for vitamin B_{12}.

Assessment of Status

Vitamin B_{12} status can be assessed in several ways. Hematological parameters (i.e., hematocrit, hemoglobin, erythrocyte count, mean corpuscular volume) can be used to indicate vitamin B_{12} status (Food and Nutrition Board 1998). Serum or plasma B_{12} concentrations are also used to examine status of vitamin B_{12}, and 170 to 250 pg/ml (120 to 180 pmol/L) is considered the lower limit for adults (Seetharam and Alpers 1982; Food and Nutrition Board 1998). However, it is possible to have normal serum concentrations of vitamin B_{12} but have low tissue status, as vitamin B_{12} is pulled from the tissues to maintain blood concentrations.

Substrates that are normally metabolized by vitamin B_{12}–dependent enzymes will be elevated in a deficient state. The concentrations of methylmalonyl CoA rise in the serum with poor vitamin B_{12} status (Food and Nutrition Board 1998). The normal range for serum methylmalonyl CoA is 73 to 271 nmol/L. Because an elevation in serum methylmalonyl CoA is highly specific to a vitamin B_{12} deficiency, serum methylmalonyl CoA concentrations are a preferred marker of B_{12} status. Urinary methylmalonyl CoA concentrations are generally minimal but in a vitamin B_{12} deficiency can reach 300 mg/day (Beck 1991; Kuzminski et al. 1998). As with folate,

there is an inverse relationship between vitamin B_{12} status and plasma homocysteine concentrations (Mason and Miller 1992; Selhub et al. 1999). Thus, plasma homocysteine concentrations can be used as a functional marker of B_{12} status. The reference value for elevated plasma homocysteine is >12 μmol/L (1.62 mg/L) (Rasmussen et al. 1996). However, >14 μmol/L (1.89 mg/L) and >16 μmol/L (2.16 mg/L) have also been used (Food and Nutrition Board 1998; Selhub et al. 1999).

Dietary Intake and Status of Vitamin B_{12} in Active Adults

Research examining the mean vitamin B_{12} intakes of female athletes is somewhat contradictory (Table 7.11). Most studies report adequate mean vitamin B_{12} intakes in female athletes (Kaiserauer et al. 1989; Keith et al. 1989; Manore et al. 1989; Nieman et al. 1989; Worme et al. 1990; Faber and Benadé 1991; Beals and Manore 1998; Kopp-Woodroff et al. 1999; Beshgetoor and Nichols 2003; Joubert and Manore 2008), while some studies report lower intakes (Leydon and Wall 2002; Clark et al. 2003). For example, Faber and Benadé (1991) examined the dietary intake of 10 female field athletes using 7-day food records. All of the participants reported consuming more than the RDA (5.26 ± 2.29 μg/day). Joubert and Manore (2008) examined differences in B-vitamin status between active and less-active women. There were no differences between the groups in dietary vitamin B_{12} intake, and all individuals in the study met the 1998 RDA for vitamin B_{12}.

Although some studies report adequate group mean dietary intakes for B_{12}, some individual athletes do not meet dietary recommendations. Dietary intakes from heavyweight female rowers ($n = 16$) were examined in a study completed by Steen et al. (1995). Participants completed 5-day food records representing the training week before competition, which were compared to the 1989 RDA. Only 80% of the athletes met the RDA for vitamin B_{12}. The authors attributed the poor intake of vitamin B_{12} to the low intake of dairy, beef, and poultry.

Some studies report lower mean dietary intakes of vitamin B_{12} in active individuals (Table 7.11). For example, female soccer players completed food records for 3 days on two occasions (pre- and postseason) (Clark et al. 2003). The mean dietary intake for vitamin B_{12} during the postseason was much lower than during the preseason (preseason 4.9 ± 1.9 μg/day; postseason 2.1 ± 1.7 μg/day). The postseason average intake was also lower than the 1998 RDA but was adequate when compared to the 1998 EAR (2.0 μg/day) (Food and Nutrition Board 1998). Similarly, Leydon and Wall (2002) reported mean dietary intakes of vitamin B_{12} from female jockeys less than the 1998 RDA but more than the EAR (female jockeys 2.15 ± 1.07 μg/day).

Studies that have examined static biochemical markers of vitamin B_{12} (i.e., plasma or serum concentrations) suggest the risk of poor status is low. For example, Singh et al. (1993) completed a study involving male and female ultramarathoners, dietary intakes, supplement use, and biochemical markers of vitamin B_{12}. Food plus supplement increased total intake of vitamin B_{12} to greater than 50 μg/day. Plasma and urinary concentrations of vitamin B_{12} were within the normal reference range. Thus, this study found adequate vitamin B_{12} intake and status in high-endurance athletes. However, Telford et al. (1992) reported that 5% of their participants (male and female athletes) had poor vitamin B_{12} status. Unfortunately, dietary intake of vitamin B_{12}

TABLE 7.11
Summary of Studies Examining Vitamin B_{12} Status in Adult Female Athletes[a]

Study	Participants	Assessment Index	Results
Beals and Manore 1998	24 female athletes with subclinical eating disorders; 24 female control athletes	7-day weighed food record; serum B_{12}	Dietary Athletes with subclinical eating disorders: 3.9 ± 2.6 μg/day Control athletes: 4.3 ± 1.9 μg/day Blood Athletes with subclinical eating disorders: 254 ± 77 pmol/L Control athletes: 331 ± 172 pmol/L
Beshgetoor and Nichols 2003	25 female master nonsupplementing cyclists and runners	4-day food record	6 ± 2 μg/day
Clark et al. 2003	13 female collegiate soccer players	Two 3-day food records (pre- and postseason)	Preseason: 4.5 ± 1.9 μg/day Postseason: 2.1 ± 1.7 μg/day
Faber and Benadé 1991	30 field athletes (discus, hammer, and javelin throwers; shotputters) (10 female)	7-day food record	Female: 5.26 ± 2.29 μg/day
Joubert and Manore 2008	64 recreational athletes (38 female)	7-day weighed food records; plasma B_{12}; plasma homocysteine	Female low physical activity: Vitamin B_{12}: 5.3 ± 4.8 μg/day Plasma B_{12}: 395 ± 162 pmol/L Plasma homocysteine: 7.4 ± 1.6 μmol/L Female high physical activity: Vitamin B_{12}: 5.3 ± 2.5 μg/day Plasma B_{12}: 357 ± 159 pmol/L Plasma homocysteine: 7.4 ± 1.1 μmol/L

Kaiserauer et al. 1989	17 female distance runners (8 with amenorrhea; 9 with eumenorrhea)	3-day food record	Amenorrheic distance runners: 2.8 µg/day (mean) Eumenorrheic distance runners: 4.1 µg/day (mean)
Keith et al. 1989	8 female highly trained cyclists	3-day weighed food record	3.3 ± 4.7 µg/day
Kopp-Woodroffe et al. 1999	4 female amenorrheic runners	7-day weighed food record; plasma B_{12}	B_{12}: 3.2 ± 1.1 µg/day Plasma B_{12}: 298 ± 43 pg/ml
Leydon and Wall 2002	19 jockeys (14 female)	7-day weighed food record	Female: 2.15 ± 1.07 µg/day
Manore et al. 1989	10 female long-distance runners	3-day food record	2.5 ± 1.3 µg/day
Nieman et al. 1989	347 marathon runners (56 female)	3-day food record	Female: 2.98 ± 3.32 µg/day
Telford et al. 1992	86 athletes (36 female)	Plasma B_{12}	Female: 299 ± 35 pmol/L
Worme et al. 1990	71 triathletes (21 female)	3-day food record	Female: 4.9 ± 4.6 µg/day

Source: Adapted from Woolf, K., and Manore, M. M., 2006, B Vitamins and Exercise: Does Exercise Alter Requirement, *International Journal of Sport Nutrition and Exercise Metabolism* 16: 453–484. With permission.

[a] Values reported as mean ± standard deviation unless noted.

was not included in this study. Overall, the risk of poor vitamin B_{12} status is low in active individuals when adequate energy and animal products are consumed and supplementation is occurring.

However, studies using functional markers of vitamin B_{12} (i.e., methylmalonic acid, plasma homocysteine) have found mixed results. Nygård et al. (1995) reported that highly active individuals (men and women) had significantly lower homocysteine concentrations than their sedentary counterparts. However, this study did not report B-vitamin intake of the research participants. Herrmann et al. (2005) examined B_{12} status of endurance athletes and sedentary controls. Although there were no differences between groups in the blood nutrient concentrations, the endurance athletes had significantly higher median serum methylmalonic acid and lower plasma homocysteine concentrations than the controls, leading the authors to speculate that recreational athletes may have altered vitamin B_{12} metabolism. Recently, Joubert and Manore (2008) examined differences in B-vitamin status and plasma homocysteine concentrations in active and less-active females. When controlling for plasma B-vitamin concentrations, the plasma homocysteine concentrations were higher in the active versus the less-active individuals. A recent study documented the effect of acute strenuous endurance exercise in male and female recreational athletes on blood homocysteine concentrations (Herrmann et al. 2003); marathon running induced a significant increase in homocysteine concentrations, while mountain biking (120 km) or running (100 km) had no impact. More research is needed to determine the impact of physical activity on homocysteine concentrations and B_{12} status in active individuals, especially women.

Vitamin B_{12} and Performance

Currently there is no research documenting the effect of vitamin B_{12} supplementation on performance in female athletes. However the research literature to date indicates that supplementation with vitamin B_{12} in well-nourished male athletes does not improve performance (Tin-May-Than et al. 1978; Read and McGuffin 1983).

SUMMARY AND RECOMMENDATIONS

The B-vitamins play important roles in the health and exercise performance of the female athlete. To obtain adequate status of the B-vitamins, female athletes need to include nutrient-dense foods and adequate energy to maintain weight and meet the energy demands of physical activity. Athletes with poor nutritional status for a B-vitamin will have a decrease in exercise performance. For female athletes with good nutritional status, B-vitamin supplementation will not improve performance. Unfortunately, research examining B-vitamin status under controlled feeding conditions is limited in female athletes. The current research suggests risk of poor thiamin status in active women is low because only a small number of active individuals have been reported to have poor thiamin status. Carefully controlled feeding studies show that exercise increases riboflavin requirements in young and old physically active women, especially if individuals are dieting for weight loss. Thus, active women, especially those dieting for weight loss, need to meet or exceed the current RDA for riboflavin on a daily basis. It is well documented that

exercise may increase the loss of vitamin B_6 through urinary 4-pyridoxic acid, but the increased losses can easily be met through nutrient-dense food choices. The fortification of grains with folic acid has decreased the risk of poor folate status, but highly active women may still be at risk for poor folate status due to low dietary folate intakes. Adequate information is not available to determine whether exercise and physical activity increase the need for niacin, pantothenic acid, biotin, choline, folate, and vitamin B_{12} in active women. If active women restrict dietary intakes or make poor food choices, they will increase their risk of poor status of the B-vitamins. These women will benefit from vitamin supplementation at intakes similar to the RDA.

REFERENCES

Barr, S. I., and Rideout, C. A. 2004. Nutritional considerations for vegetarian athletes. *Nutrition* 20:696–703.

Bayliss, R. M., Brookes, R., McCulloch, J. M., Kuyl, J., and Metz, J. 1984. Urinary thiamine excretion after oral physiological doses of vitamin. *International Journal for Vitamin and Nutrition Research* 54:161–164.

Beals, K. A., and Manore, M. M. 1998. Nutritional status of female athletes with subclinical eating disorders. *Journal of the American Dietetic Association* 98(4):419–425.

Beals, K. A., and Manore, M. M. 2007. Nutritional considerations for the female athlete. In: *Advances in sport and exercise science series: Nutrition and sport*, ed. N. Spurway, and D. MacLaren, 187–206. Philadelphia, PA: Elsevier.

Beck, W. 1991. Neuropsychiatric consequences of cobalamin deficiency. *Advances in Internal Medicine* 36:33–36.

Belko, A. Z., Meredith, M. P., Kalkwarf, H. J., Obarzanek, E., Weinberg, S., Roach, R., McKeon, G., and Roe, D. A. 1985. Effects of exercise on riboflavin requirements: Biological validation in weight reducing women. *American Journal of Clinical Nutrition* 41:270–277.

Belko, A. Z., Obarzanek, E., Kalkwarf, H. J., Rotter, M. A., Bogusz, S., Miller, D., Haas, J. D., and Roe, D. A. 1983. Effects of exercise on riboflavin requirements of young women. *American Journal of Clinical Nutrition* 37:509–517.

Belko, A. Z., Obarzanek, E., Roach, R., Rotter, M., Urban, G., Weinberg, S., and Roe, D. A. 1984. Effects of aerobic exercise and weight loss on riboflavin requirements of moderately obese, marginally deficient young women. *American Journal of Clinical Nutrition* 40:553–561.

Beshgetoor, D., and Nichols, J. F. 2003. Dietary intake and supplement use in female master cyclists and runners. *International Journal of Sport Nutrition and Exercise Metabolism* 13(2):166–172.

Borrione, P., Pigozzi, F., Massazza, G., Schonhuber, H., Viberti, G., Paccotti, P., and Angeli, A. 2007. Hyperhomocysteinemia in winter elite athletes: A longitudinal study. *Journal of Endocrinological Investigation* 30:367–375.

Buchman, A. L., Awal, M., Jenden, D., Roch, M., and Kang, S. H. 2000. The effect of lecithin supplementation on plasma choline concentrations during a marathon. *Journal of the American College of Nutrition* 9:768–778.

Buchman, A. L., Jenden, D., and Roch, M. 1999. Plasma free, phospholipid-bound and urinary choline all decrease during a marathon run and may be associated with impaired performance. *Journal of the American College of Nutrition* 18(6):598–601.

Clark, M., Reed, D. B., Crouse, S. F., and Armstrong, R. B. 2003. Pre- and post-season dietary intake, body composition, and performance indices of NCAA Division I female soccer players. *International Journal of Sport Nutrition and Exercise Metabolism* 13(3):303–319.

Cohenour, S. H., and Calloway, D. H. 1972. Blood, urine, and dietary pantothenic acid levels of pregnant teenagers. *American Journal of Clinical Nutrition* 25:512–517.

Crozier, P. G., Cordain, L., and Sampson, D. A. 1994. Exercise-induced changes in plasma vitamin B-6 concentrations do not vary with exercise intensity. *American Journal of Clinical Nutrition* 60:552–558.

Di Santolo, M., Banfi, G., Stel, G., and Cauci, S. 2009. Association of recreational physical activity with homocysteine, folate and lipid markers in young women. *European Journal of Applied Physiology* 105:111–118.

Doyle, M. R., Webster, M. J., and Erdmann, L. D. 1997. Allithiamine ingestion does not enhance isokinetic parameters of muscle performance. *International Journal of Sport Nutrition* 7:39–47.

Faber, M., and Benadé, A. J. S. 1991. Mineral and vitamin intake in field athletes (discus-, hammer-, javelin-throwers and shotputters). *International Journal of Sports Medicine* 12(3):324–327.

Finglas, P. M. 1994. Thiamin. *International Journal for Vitamin and Nutrition Research* 63:270–274.

Fogelholm, M. 1992. Micronutrient status in females during a 24-week fitness-type exercise program. *Annals of Nutrition & Metabolism* 36(4):209–218.

Fogelholm, M., Rehunen, S., Gref, C. G., Laakso, J. T., Lehto, J., Ruokonen, I., and Himberg, J. J. 1992. Dietary intake and thiamin, iron, and zinc status in elite Nordic skiers during different training periods. *International Journal of Sport Nutrition* 2(4):351–365.

Fogelholm, M., Ruokonen, I., Laakso, J. T., Vourimaa, T., and Himberg, J. J. 1993. Lack of association between indices of vitamin B-1, B-2 and B-6 status and exercise-induced blood lactate in young adults. *International Journal of Sport Nutrition* 3:165–176.

Food and Nutrition Board. Institute of Medicine. 1998. *Dietary reference intakes: Thiamin, riboflavin, niacin, vitamin B-6, folate, vitamin B-12, pantothenic acid, biotin, and choline*. Washington, DC: National Academy Press.

Gibson, R. S. 2005. *Principles of nutritional assessment*, 2nd ed. New York: Oxford University Press.

Gregory, J. F. 1997. Bioavailability of folate. *European Journal of Clinical Nutrition* 51(1):S54–S59.

Gropper, S. S., Smith, J. L., and Gropper, J. L. 2009. The water-soluble vitamins. In: *Advanced nutrition and human nutrition,* 5th ed., 309–372. Belmont, CA: Thomson Wadsworth Learning.

Hansen, C. M., and Manore, M. M. 2005. Vitamin B6. In: *Sports nutrition: Vitamin and trace elements*, 2nd ed., ed. J. A. Driscoll and I. Wolinsky, 81–91. Boca Raton, FL: CRC Press.

Hawley, J. A., Dennis, S. C., Lindsay, F. H., and Noakes, T. D. 1995. Nutritional practices of athletes: Are they sub-optimal? *Journal of Sports Sciences* 13:S75–S81.

Herbert, V., and Das, K. C. 1994. Folic acid and vitamin B12. In: *Modern nutrition in health and disease*, 8th ed., ed. M. E. Shils, J. A. Olson, and M. Shike, 402–425. Philadelphia, PA: Lea & Febiger.

Herrmann, M., Obeid, R., Scharhag, J., Kindermann, W., and Herrmann, W. 2005. Altered vitamin B-12 status in recreational endurance athletes. *International Journal of Sports Nutrition and Exercise Metabolism* 15:433–441.

Herrmann, M., Schorr, H., Obeid, R., Scharhag, J., Urhausen, A., Kindermann, W., and Herrmann, W. 2003. Homocysteine increases during endurance exercise. *Clinical Chemistry and Laboratory Medicine* 41(11):1518–1524.

Hoch, A. Z., Lynch, S. L., Jurva, J. W., Schimke, J. E., and Gutterman, D. D. 2010. Folic acid supplementation improves vascular function in amenorrheic runners. *Clinical Journal of Sport Medicine* 20:205–210.

Hofmann, A., Reynolds, R. D., Smoak, B. L., Villanueva, V. G., and Deuster, P. A. 1991. Plasma pyridoxal and pyridoxal 5′-phosphate concentrations in response to ingestion of water or glucose polymer during a 2-h run. *American Journal of Clinical Nutrition* 53:84–89.

Joubert, L. M., and Manore, M. M. 2008. The role of physical activity level and B-vitamin status on blood homocysteine levels. *Medicine and Science in Sports and Exercise* 40(11):1923–1931.

Kaiserauer, S., Snyder, A. C., Sleeper, M., and Zierath, J. 1989. Nutritional, physiological, and menstrual status of distance runners. *Medicine and Science in Sports and Exercise* 21(2):120–125.

Keith, R. E., and Alt, L. A. 1991. Riboflavin status of female athletes consuming normal diets. *Nutrition Research* 11:727–734.

Keith, R. E., O'Keeffe, K. A., Alt, L. A., and Young, K. L. 1989. Dietary status of trained female cyclists. *Journal of the American Dietetic Association* 89(11):1620–1623.

Kopp-Woodroffe, S. A., Manore, M. M., Dueck, C. A., Skinner, J. S., and Matt, K. S. 1999. Energy and nutrient status of amenorrheic athletes participating in a diet and exercise training intervention program. *International Journal of Sport Nutrition* 9:70–88.

Kuzminski, A. M., Del Giacco, E. S., Allen, R. H., Stabler, S. P., and Lindenbaum, J. 1998. Effective treatment of cobalamin deficiency with oral cobalamin. *Blood* 92:1191–1198.

Lawrence, J. D., Smith, J. L., Bower, R. C., and Riehl, W. P. 1975. The effect of alpha tocopherol (vitamin E) and pyridoxine HCL (vitamin B6) on the swimming endurance of trained swimmers. *Journal of the American College Health Association* 23(3):219–222.

Leklem, J. E. 1990. Vitamin B-6: A status report. *Journal of Nutrition* 120:1503–1507.

Leklem, J. E., and Shultz, T. D. 1983. Increased plasma pyridoxal 5′-phosphate and vitamin B6 in male adolescents after a 4500-meter run. *American Journal of Clinical Nutrition* 38:541–548.

Leydon, M. A., and Wall, C. 2002. New Zealand jockeys' dietary habits and their potential impact on health. *International Journal of Sport Nutrition and Exercise Metabolism* 12(2):220–237.

Lui, A., Lumeng, L., Aronoff, G. R., and Li., T. K. 1985. Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: Metabolic balance studies in humans. *Journal of Laboratory and Clinical Medicine* 106:491–497.

Lukaski, H. C. 2004. Vitamin and mineral status: Effects on physical performance. *Nutrition* 20:632–644.

Manore, M. M. 1994. Vitamin B-6 and exercise. *International Journal of Sport Nutrition* 4(2):89–103.

Manore, M. M. 1999. Nutritional needs of the female athlete. In: *Clinics in sports medicine: Nutritional aspects of exercise,* ed. K. B. Wheeler, and J. A. Lombardo, 549–563. Philadelphia, PA: W.B. Saunders.

Manore, M. M. 2000. Effect of physical activity on thiamine, riboflavin, and vitamin B6 requirements. *American Journal of Clinical Nutrition* 72:598S–606S.

Manore, M. M., Barr, S. I., and Butterfield, G. E. 2000. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the American Dietetic Association* 100:1543–1556.

Manore, M. M., Besenfelder, P. D., Wells, C. L, Carroll, S. S., and Hooker, S. P. 1989. Nutrient intakes and iron status in female long-distance runners during training. *Journal of the American Dietetic Association* 89:257–259.

Manore, M. M., and Leklem, J. E. 1988. Effect of carbohydrate and vitamin B6 on fuel substrates during exercise in women. *Medicine and Science in Sports and Exercise* 20(3):233–241.

Manore, M. M., Leklem, J. E., and Walter, M. C. 1987. Vitamin B-6 metabolism as affected by exercise in trained and untrained women fed diets differing in carbohydrate and vitamin B-6 content. *American Journal of Clinical Nutrition* 46:995–1004.

Manore, M. M., Meyer, N. L., and Thompson, J. 2009. *Sport nutrition for health and performance,* 2nd ed., 275–301. Champaign, IL: Human Kinetics.

Mason, J. B., and Miller, J. W. 1992. The effects of vitamins B12, B6, and folate on blood homocysteine levels. *Annals of the New York Academy of Sciences* 669:197–203.

Matter, M., Stittfall, T., Graves, J., Myburgh, K., Adams, B., Jacobs, P., and Noakes, T. D. 1987. The effect of iron and folate therapy on maximal exercise performance in female marathon runners with iron and folate deficiency. *Clinical Science* 72:415–422.

Maughan, R. J. 1999. Role of micronutrients in sport and physical activity. *British Medical Bulletin* 55(3):683–690.

Murray, R., Bartoli, W. P., Eddy, D. E., and Horn, M. K. 1995. Physiological and performance responses to nicotinic-acid ingestion during exercise. *Medicine and Science in Sports and Exercise* 27(7):1057–1062.

Nieman, D. C., Butler, J. V., Pollett, L. M., Dietrich, S. J., and Lutz, R. D. 1989. Nutrient intake of marathon runners. *Journal of the American Dietetic Association* 89(9):1273–1278.

Nygård, O., Vollset, S. E., Refsum, H., Stensvold, I., Tverdal, A., Nordrehaug, J. E., Ueland, P. M., and Kvåle, G. 1995. Total plasma homocysteine and cardiovascular risk profile: The Hordaland Homocysteine Study. *Journal of the American Medical Association* 274(19):1526–1533.

Pancharuniti, N., Lewis, C. A., Sauberlich, H. E., Perkins, L. L, Go, R. C. P., Alvarez, J. O., Macaluso, M., Acton, R. T., Copeland, R. B., Cousins, A. L., Gore, T. B., Cornwell, P. E., and Roseman, J. M. 1994. Plasma homocyst(e)ine, folate, and vitamin B-12 concentrations and risk for early-onset coronary artery disease. *American Journal of Clinical Nutrition* 59:940–948.

Papapanagiotou, A., Gissis, I., Papadopoulos, C., Souglis, A., Bogdanis, G. C., Giosos, I., and Sotiropoulos, A. 2011. Changes in homocysteine and 8-iso-PGF_{2a} levels in football and hockey players after a match. *Research in Sports Medicine* 19:118–128.

Penry, J. T., and Manore, M. M. 2008. Choline: An important micronutrient for maximal endurance-exercise performance? *International Journal of Sport Nutrition and Exercise Metabolism* 18:191–203.

Raczynski, G., and Szczepanska, B. 1993. Longitudinal studies on vitamin B-1 and B-6 status in Polish elite athletes. *Biology of Sport* 10(3):189–194.

Rankinen, T., Lyytikainen, S., Vanninen, E., Penttila, I., Rauramaa, R., and Uusitupa, M. 1998. Nutritional status of the Finnish elite ski jumpers. *Medicine and Science in Sports and Exercise* 30(11):1592–1597.

Rasmussen, K., Møller, J., Lyngbak, M., Pedersen, A., and Dybkjaer, L. 1996. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. *Clinical Chemistry* 42(4):630–636.

Read, M. H., and McGuffin, S. L. 1983. The effect of B-complex supplementation on endurance performance. *Journal of Sports Medicine and Physical Fitness* 23(2):178–184.

Rokitzki, L., Sagredos, A., Keck, E., Sauer, B., and Keul, J. 1994a. Assessment of vitamin B-2 status in performance athletes of various types of sports. *Journal of Nutritional Science and Vitaminology* 40:11–22.

Rokitzki, L., Sagredos, A. N., Reuss, F., Buchner, M., and Keul, J. 1994b. Acute changes in vitamin B6 status in endurance athletes before and after a marathon. *International Journal of Sport Nutrition* 4:154–165.

Rokitzki, L., Sagredos, A. N., Reuss, F., Cufi, D., and Keul, J. 1994c. Assessment of vitamin B-6 status of strength and speedpower athletes. *Journal of the American College of Nutrition* 13(1):87–94.

Sato, A., Shimoyama, Y., Ishikawa, T., and Murayama, N. 2011. Dietary intake and blood thiamin and riboflavin concentrations in college swimmers undergoing intensive training. *International Journal of Sport Nutrition and Exercise Metabolism* 21:195–204.

Sauberlich, H. 1999. Pantothenic acid. In: *Laboratory tests for the assessment of nutritional status,* 2nd ed., pp. 175–183. Boca Raton, FL: CRC Press.

Savage, D. G., and Lindenbaum, J. 1995. Folate-cobalamin interactions. In: *Folate in health and disease,* ed. L. B. Bailey, 237–285. New York: Marcel Dekker.

Seetharam, B., and Alpers, D. H. 1982. Absorption and transport of cobalamin (vitamin B12). *Annual Review of Nutrition* 2:343–369.

Selhub, J., Jacques, P. F., Rosenberg, I. H., Rogers, G., Bowman, B. A., Gunter, E. W., Wright, J. D., Johnson, C. L. 1999. Serum total homocysteine concentrations in the Third National Health and Nutrition Examination Survey (1991–1994): Population reference ranges and contribution of vitamin status to high serum concentrations. *Annals of Internal Medicine* 131(5):331–339.

Selhub, J., Jacques, P. F., Wilson, P. W. F., Rush, D., and Rosenberg, I. H. 1993. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *Journal of the American Medical Association* 270(22):2693–2698.

Shibata, K., and Matsuo, H. 1989. Effect of supplementing low protein diets with the limiting amino acids on the excretion of N′ methylnicotinamide and its pyridones in rats. *Journal of Nutrition* 119:896–901.

Singh, A., Evans, P., Gallagher, K. L., and Deuster, P. A. 1993. Dietary intakes and biochemical profiles of nutritional status of ultramarathoners. *Medicine and Science in Sports and Exercise* 25(3):328–334.

Spodaryk, K., Czekaj, J., and Sowa, W. 1996. Relationship among reduced level of stored iron and dietary iron in trained women. *Physiological Research* 45:393–397.

Steen, S. N., Mayer, K., Brownell, K. D., and Wadden, T. A. 1995. Dietary intake of female collegiate heavyweight rowers. *International Journal of Sport Nutrition* 5:225–231.

Subotičanec, K., Stavljenić, A., Schalch, W., and Buzina, R. 1990. Effects of pyridoxine and riboflavin supplementation on physical fitness in young adolescents. *International Journal for Vitamin and Nutrition Research* 60(1):81–88.

Tarr, J. B., Tamura, T., and Stokstad, E. L. R. 1981. Availability of vitamin B6 and pantothenate in an average American diet in man. *American Journal of Clinical Nutrition* 34:1328–1337.

Telford, R. D., Catchpole, E. A., Deakin, V., McLeay, A. C., and Plank, A. W. 1992. The effect of 7 to 8 months of vitamin/mineral supplementation on the vitamin and mineral status of athletes. *International Journal of Sport Nutrition* 2:123–134.

Tin-May-Than, Ma-Win-May, Khin-Sann-Aung, and Mya-Tu, M. 1978. The effect of B12 on physical performance capacity. *British Journal of Nutrition* 40(2):269–273.

van der Beek, E. J., van Dokkum, W., Wedel, M., Schrijver, J., and van den Berg, H. 1994. Thiamin, riboflavin and vitamin B-6: Impact of restricted intake on physical performance in man. *Journal of the American College of Nutrition* 13(6):629–640.

Volpe, S. L. 2007. Micronutrient requirements for athletes. *Clinics in Sports Medicine* 26:119–130.

Von Allwörden, H. N, Horn, S., Kahl, J., and Feldheim, W. 1993. The influence of lecithin on plasma choline concentrations in triathletes and adolescent runners during exercise. *European Journal of Applied Physiology and Occupational Physiology* 67:87–91.

Warnock, L. G., Prudhomme, C. R., and Wagner, C. 1979. The determination of thiamin pyrophosphate in blood and other tissues, and its correlation with erythrocyte transketolase activity. *Journal of Nutrition* 108:421–427.

Webster, M. J.1998. Physiological and performance responses to supplementation with thiamin and pantothenic acid derivatives. *European Journal of Applied Physiology and Occupational Physiology* 77:486–491.

Webster, M. J., Scheett, T. P., Doyle, M. R., and Branz, M. 1997. The effect of a thiamin derivative on exercise performance. *European Journal of Applied Physiology and Occupational Physiology* 75:520–524.

Weight, L. M, Myburgh, K. H., and Noakes, T. D. 1988. Vitamin and mineral supplementation: Effect on the running performance of trained athletes. *American Journal of Clinical Nutrition* 47:192–195.

Wildman, R. E. C., and Miller, B. S. 2004. Vitamins and exercise. In: *Sports and fitness nutrition*, 252–293. Belmont, CA: Wadsworth.

Winters, L. R. T., Yoon, J. S., Kalkwarf, H. J., Davies, J. C., Berkowitz, M. G., Haas, J., and Roe, D. A. 1992. Riboflavin requirements and exercise adaptation in older women. *American Journal of Clinical Nutrition* 56:526–532.

Wittwer, C. T., Schweitzer, C., Pearson, J., Song, W. O, Windham, C. T., Wyse, B. W., and Hansen, R. G. 1989. Enzymes for liberation of pantothenic acid in blood: Use of plasma pantetheinase. *American Journal of Clinical Nutrition* 50:1072–1078.

Woolf, K., and Manore, M. M. 2006. B-vitamins and exercise: Does exercise alter requirement. *International Journal of Sport Nutrition and Exercise Metabolism* 16:453–484.

Woolf, K., and Manore, M. M. 2007. Micronutrients important for exercise. In: *Advances in sport and exercise science series: Nutrition and sport*, ed. N. Spurway, and D. MacLaren, 119–136. Philadelphia, PA: Elsevier.

Worme, J. D., Doubt, T. J., Singh, A., Ryan, C. J., Moses, F. M., and Deuster, P. A. 1990. Dietary patterns, gastrointestinal complaints, and nutrition knowledge of recreational triathletes. *American Journal of Clinical Nutrition* 51:690–697.

Ziesel, S. H. 2000. Choline: An essential nutrient for humans. *Nutrition* 16(7/8):669–671.

Ziesel, S. H., da Costa, K., Franklin, P. D., Alexander, E. A., Lamont, J. T., Sheard, N. F., and Beiser, A. 1991. Choline, an essential nutrient for humans. *FASEB Journal* 5:2093–2098.

Ziesel, S. H., Growden, J. H., Wurtman, R. J., Magil, S. G., and Logue, M. 1980. Normal plasma choline responses to ingested lecithin. *Neurology* 30:1226–1229.

8 Introduction: The Female Athlete Triad—*Energy Availability, Menstrual Function, and Bone Health*

Katherine A. Beals

The health benefits of regular physical activity for women are well documented. And the opportunities for women to participate in sports and reap those benefits are currently unprecedented. Nonetheless, for a small percentage of female athletes, the desire for athletic success combined with the pressure to achieve a prescribed body weight may lead to the development of a triad of disorders including low energy availability, menstrual dysfunction, and low bone mineral density (BMD)—known collectively as the *Female Athlete Triad* (Otis et al. 1997; Nattiv et al. 2007). Alone or in combination, the disorders of the female athlete triad can negatively impact both the health and the physical performance of the female athlete.

The triad was first formally described in 1992 when a special American College of Sports Medicine (ACSM) *Task Force on Women's Issues* convened a consensus conference to discuss the incidence of a triad of disorders—disordered eating, amenorrhea, and osteoporosis—afflicting female athletes with increasing frequency. This combination of disorders was subsequently given the formal name of the *Female Athlete Triad* (hereafter, the Triad). In 1997, the ACSM published a Position Stand, which not only documented the prevalence and consequences of the individual disorders of the Triad, but called for further research into the causes, prevention, and treatment of the Triad as a whole (Otis et al. 1997). Ten years later, a second Position Stand by the ACSM was released with updated research and new recommendations regarding the Triad (Nattiv et al. 2007). In addition, the Triad categories were updated and renamed to better reflect the spectrum that exists for each of the disorders ranging from health to disease as opposed to focusing only on the extreme end point of each disorder. Specifically, the term *disordered eating* was replaced by *energy availability*, *amenorrhea* was replaced by *menstrual function*, and *osteoporosis* by *bone health*.

The following three chapters (Chapters 9, 10, and 11) will describe, in detail, each of the Triad categories. The authors will present the most current research regarding the prevalence and consequences of each of the Triad components as well as suggestions for interventions and treatments with the goal of safeguarding both the health and performance of the female athlete.

REFERENCES

Nattiv, A., Loucks, A. B., Manore, M. M., Sanborn, C. F., Sundgot-Borgen, J., and Warren, M. P. 2007. American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc*. 39:1867–1882.

Otis, C. L., Drinkwater, B., Johnson, M., Louks, A., and Wilmore, J. H. 1997. American College of Sports Medicine position stand. The female athlete triad: Disordered eating, amenorrhea, and osteoporosis. *Med Sci Sports Exerc*. 29:i–ix.

9 Energy Availability, Health, and Performance in the Female Athlete

Katherine A. Beals

CONTENTS

INTRODUCTION

In the newly revised *Female Athlete Triad* (the *Triad*) position stand, the American College of Sports Medicine (ACSM) asserts that low energy availability is the cornerstone of the metabolic and health consequences associated with the Triad; in other words, it is at the root of both menstrual dysfuction and poor bone health. Energy availability is defined as the amount of energy available for the metabolic processes of the body *after* energy is used for exercise, normalized for fat-free mass (FFM) (i.e., Energy availability = Energy intake – Energy expenditure per kilogram of fat-free mass) (Nattiv et al. 2007). Although low energy availability can (and often does) result from disordered eating, it may also result from the athlete inadvertently failing to meet exercise energy requirements due to, among other reasons, time constraints, food availability issues, and lack of appropriate nutrition knowledge, particularly as it relates to body weight management (Nattiv et al. 2007).

This chapter will define the concept of energy availability, describe the currently accepted energy avialability categories, and summarize the existing research regarding the etiology, prevalence, and consequences of low energy availability among female athletes. Suggestions for correcting low energy availability in female athletic populations will also be discussed.

THE CONCEPT OF ENERGY AVAILABILITY

Energy availability (EA) is a distinct concept from energy balance and thus needs to be calculated and interpreted quite differently. Energy balance (EB) is generally defined as energy intake (EI) (i.e., calories consumed) minus *total daily energy expenditure* (EE), which includes energy expended as a result of basal metabolism (BMR), the thermic effect of food (TEF), the thermic effect of physical activity (TEE), and adaptive thermogenesis (AT). The equation is most often expressed as EB = EI – EE, and the direction of the balance—EI > EE, EI < EE, or EI = EE—will determine the amount of dietary energy added to or lost from the body's energy stores and, thus, whether body weight and body composition increase, decrease, or remain the same, respectively.

In contrast, the concept of EA describes the calories available to the body to optimize its physiological and metabolic functions and characterizes the effects of changes in the availability of energy on those functions. As explained by Wade and Jones (2004), dietary energy is required for a variety of physiological processes (e.g., circulation, basic cellular maintenance, somatic growth, thermoregulation, acid-base balance, immune function, locomotion, reproduction, etc.), and energy that is expended on one of these processes is no longer available to support the others. Based on this concept, energy availability would be calculated by subtracting the energy cost of the physiological process of interest (e.g., locomotion) from the total energy intake to determine what is "left over" (i.e., available) to support the remaining physiological processes (i.e., circulation, basic cellular maintenance, growth, thermoregulation, etc.). To apply the concept of EA to the female athlete then, energy expended in physical activity would be subtracted from the total energy intake to determine the amount of energy remaining after exercise training that is "available" for all other physiological and metabolic processes. Thus, EA as a component of the Triad has been defined as dietary energy intake minus exercise energy expenditure (EEE). Because more energy is expended by fat-free mass than fat mass, it is also useful to normalize energy availability to fat-free mass so that EA = (EI – EEE)/FFM (Loucks et al. 2011). (See Box 9.1.)

Wade and Jones (2004) contend that the body's physiological and metabolic processes can be more or less prioritized in terms of their importance to individual survival. Accordingly, some physiological processes (e.g., circulation) cannot be compromised and will be maintained at all costs. Other processes can be affected to varying degrees without threatening individual survival. For example, somatic growth can be slowed during calorie deprivation without significantly affecting long-term survival, and thermoregulatory costs can be reduced by putting on a sweater or huddling under a blanket. Still other physiological and metabolic processes are not crucial for *individual* survival (e.g., reproduction) and may even be considered counterproductive when dietary energy is scarce or insufficient. Consequently, these processes would likely be the first to be compromised during times of low energy availability (Wade and Jones 2004).

ENERGY AVAILABILITY CATEGORIES

The hypothesis proposed by Wade and Jones (2004) not only helps to explain some of the most notable consequences of low energy availability seen in female athletes

BOX 9.1 ENERGY BALANCE VERSUS ENERGY AVAILABILITY

The following example serves to highlight the difference between energy balance and energy availability.

> Ten healthy, moderately active women are placed in a metabolic ward for 7 days where food is provided to them and their energy expenditure is carefully measured in a whole-room calorimeter. Energy requirements to maintain body weight are calculated to be 2500 kcal/day. The women are placed on a hypocaloric diet providing 2000 kcal/day; thus, they are in a negative energy balance of –500 kcal/day. Exercise energy expenditure is estimated to be 400 kcal/day, and energy availability (EA) is determined to be ~30 kcal·kg^{-1} FFM·day^{-1}. Over the course of those 7 days, EA will remain constant at 30 kcal·kg^{-1} FFM·day^{-1}. However, the negative energy balance will not remain constant. In fact, the degree of negative energy balance will begin to decrease as the various components of energy expenditure begin to decrease in response to the lower energy intake (i.e., decreased BMR, lower TEF). In contrast, EA will not change. Thus, because physiological processes are suppressed by low energy intake, measurements of energy balance may not accurately assess energy requirements for optimizing physiological function.

(i.e., infertility); but, it also serves as the basis for the development of the currently accepted energy availability categories. In a series of studies, Anne Loucks and colleagues (Loucks et al. 1998; Loucks and Thuma 2003) not only demonstrated the effects of low energy availability on markers of reproductive function, but also identified a possible minimal "threshold" of EA required for reproductive and bone health.

In the first experiment (Loucks et al. 1998), healthy, sedentary women were exposed in random order to one of four EA "conditions" (two that provided adequate EA and two that provided inadequate EA) by manipulating either energy intake or exercise energy expenditure. In two of the conditions, subjects exercised (creating a significant exercise energy expenditure) and were either given additional calories to cover the cost of the exercise energy expenditure, thus placing them in a state of adequate EA (i.e., 45 kcal·kg^{-1} FFM·day^{-1}), or not provided those additional calories, thus placing them in a state of low EA (i.e., 10 kcal·kg^{-1} FFM·day^{-1}). In the other two conditions, EA was manipulated not via exercise but rather by calorie restriction. That is, subjects were either provided with the number of calories they needed to be in energy balance (i.e., 45 kcal·kg^{-1} FFM·day^{-1}) or given a calorie restriction to put them in a state of negative energy balance and low EA (i.e., 10 kcal·kg^{-1} FFM·day^{-1}). The results indicated that in both the sedentary and exercising conditions, low EA produced significant alterations in luteinizing hormone (LH) pulsatility (indicative of a disruption in reproductive function) not seen in the adequate EA conditions. Interestingly, the disruptive effects of low EA in the exercise condition were actually smaller than those seen when the low EA was brought about via calorie restriction (Loucks et al. 1998). This study was significant not only because it clearly demonstrated the effects of low EA on reproductive function in humans, but because it was the first study to show that low EA and not the "stress" of exercise causes the

disruptions in the markers of menstrual function. (See Chapter 10 for more on menstrual dysfunction in female athletes.)

Unfortunately, the methodology employed by the study described above did not allow for a determination of the *critical* energy deficit level (i.e., the threshold of energy availability). Thus, a follow-up study was conducted in which 29 regularly menstruating, sedentary young women were placed on an exercise program designed to achieve an exercise energy expenditure of 15 kcal·kg^{-1} FFM·day^{-1} (Loucks and Thuma 2003), while consuming a diet designed to achieve EAs of 10, 20, 30, or 45 kcal·kg^{-1} FFM·day^{-1}. A variety of reproductive hormones were measured including LH, follicular stimulating hormone (FSH), and estradiol. The results indicated that LH pulsatility decreased significantly when EA dropped below 30 kcal·kg^{-1} FFM·day^{-1}, whereas LH pulse amplitude increased.

Research has also demonstrated that markers of bone turnover (i.e., bone resorption and bone formation) are significantly affected by low energy availability, and the critical threshold appears to be <30 kcal·kg^{-1} FFM·day^{-1} (Zanker and Swaine 1998; Ihle and Loucks 2004). Using a similar protocol to those used previously by their lab (and described in the preceding paragraphs), Ihle and Loucks assessed the effects of varying levels of EA on markers of bone resorption and bone formation in 29 regularly menstruating, sedentary young women. Subjects were placed on an exercise program and then provided a control diet to elicit varying levels of EA (i.e., 45, 30, 20, or 10 kcal·kg^{-1} FFM·day^{-1}) in random order. Markers of bone formation and bone resorption were measured on the different EA levels. The results indicated that markers of bone resorption were increased when EA dipped to 10 kcal·kg^{-1} FFM·day$^{-1,}$ while markers of bone formation were suppressed at all EA levels <30 kcal·kg^{-1} FFM·day^{-1}. The authors concluded that the uncoupling of bone resorption and formation by severely reduced energy availability, if left to continue, may lead to irreversible reductions in bone mineral density (BMD), and the suppression of bone formation by less severe restrictions may prevent young women from achieving their genetic potential for peak bone mass.

Based on the data described above, it has been suggested that EA <30 kcal·kg^{-1} FFM·day^{-1} be considered "low" EA, while an EA ≥45 kcal·kg^{-1} FFM·day^{-1} be considered "adequate" or "optimal" EA, leaving everything in between as a bit of gray area (Manore et al. 2007; Nattiv et al. 2007). However, it should be emphasized that these data were derived from studies that only examined the *acute* effects of low EA on *markers* of reproductive function and bone turnover in habitually *sedentary* women. It remains to be seen if alterations in these surrogate markers as a result of low EA translate into an increased risk for the disorders they are meant to represent—that is, menstrual dysfunction (amenorrhea, annovulation, luteal suppression), low BMD, and stress fracture incidence among female athletes. It is also currently not known if the EA categories derived from the markers of reproductive function and bone turnover correspond to the disorders they are indirectly measuring (i.e., menstrual dysfunction, low BMD, and fracture risk).

Despite a lack of direct evidence, indirect evidence suggests that low EA can negatively impact both menstrual function and bone health in female athletes. For example, reexamination of published reports describing energy intake and menstrual function in female endurance athletes indicates that the EA of those reporting

amenorrhea is consistently <30 kcal·kg^{-1} FFM·day^{-1} (Manore et al. 2007). And, research indicates that BMD is often lower in amenorrheic athletes than in their eumenorrheic counterparts (Drinkwater et al. 1984; Marcus et al. 1985; Myburgh et al. 1993; Rencken et al. 1996; Nichols and Sanborn 2007). In the nutritional intervention studies published to date, menstrual cycles were restored in amenorrhoeic runners by increasing their energy availability to >30 kcal·kg^{-1} FFM·day^{-1} (Kopp-Woodroffe et al. 1999; Gruebels et al. 2011). It should be noted, however, that low EA has also been shown in eumenorrheic athletes (Marcus et al. 1985; De Souza et al. 1998; Winters-Stone and Snow 2004), but in many of those studies, the methodology did not allow for the examination of more subclinical and not overtly noticeable menstrual disorders (e.g., anovulation, shortened luteal phase, etc.) (De Souza et al. 1998).

In a recent study, Beals et al. (2012) examined the associations between EA, menstrual dysfunction (MD), and compromised bone health (low BMD and stress fracture incidence) among 40 female endurance athletes (mean age 27 ± 5 years). Using the established EA categories (i.e., low <30 kcal·kg^{-1} FFM·day^{-1} and adequate ≥45 kcal·kg^{-1} FFM·day^{-1}), the researchers found that there were no associations between low EA and history of amenorrhea, menstrual irregularity, low BMD, or stress fracture incidence.

The inconsistencies in the studies described above highlight the need for more research to validate the existing EA categories as well as to determine the long-term effects of varying levels of EA on reproductive function and bone health in female athletes.

THE ETIOLOGY OF LOW ENERGY AVAILABILITY AMONG FEMALE ATHLETES

Low EA in an athlete can result from increasing exercise energy expenditure without also increasing energy intake (to cover the additional energy expended in exercise) or by reducing energy intake without concurrently reducing exercise energy expenditure. The underlying causes for the above scenarios are likely many and diverse; however, it has been suggested that they can be generally placed into one of three categories: (1) disordered eating, (2) intentional and rational but mismanaged efforts to reduce body weight, (3) inadvertent failure to increase energy intake to compensate for increased exercise energy expenditure (Nattiv et al. 2007; Loucks et al. 2011). Each of these potential causes of low EA will be discussed in greater detail below.

Disordered eating is a general term used to describe a range of extreme, unhealthy, and sometimes psychopathological behaviors aimed at reducing body weight or maintaining an extremely low body weight. Included under the general term *disordered eating* are the clinical eating disorders—anorexia nervosa and bulimia nervosa—as well as subclinical variants of these (APA 1994) (Table 9.1).

To be diagnosed with a *clinical* eating disorder, an individual must meet a standard set of criteria outlined in the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (*DSM-IV*) (APA 1994). It should be emphasized that clinical eating disorders are psychiatric conditions and thus go beyond simple body weight and shape dissatisfaction and involve more than just abnormal eating patterns and pathogenic weight control behaviors. Individuals with clinical eating disorders often display severe feelings of insecurity and worthlessness, have trouble identifying and

TABLE 9.1
***DSM IV* Diagnostic Criteria for Anorexia Nervosa and Bulimia Nervosa**

Anorexia Nervosa	Bulimia Nervosa
Refusal to maintain body weight at or above a minimally normal weight for age and height, for example, weight loss leading to maintenance of body weight less than 85% of that expected or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected.	Recurrent episodes of binge eating characterized by both (1) eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances; and (2) a sense of lack of control over eating during the episode (such as a feeling that one cannot stop eating or control what or how much one is eating).
Intense fear of gaining weight or becoming fat, even though underweight.	Recurrent inappropriate compensatory behavior to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
Disturbance in the way one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.	The binge-eating and inappropriate compensatory behavior both occur, on average, at least twice a week for 3 months.
In postmenarchal females, amenorrhea (i.e., the absence of at least three consecutive menstrual cycles). A woman having periods only while on hormone medication (e.g., estrogen) still qualifies as having amenorrhea.	Self-evaluation is unduly influenced by body shape and weight.
	The disturbance does not occur exclusively during episodes of anorexia nervosa.
Type	**Type**
Restricting Type: During the current episode of anorexia nervosa, the person has not regularly engaged in binge-eating or purging behavior (self-induced vomiting or misuse of laxatives, diuretics, or enemas).	*Purging Type*: During the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.
Binge Eating/Purging Type: During the current episode of anorexia nervosa, the person has regularly engaged in binge-eating or purging behavior.	*Nonpurging Type*: During the current episode of bulimia nervosa, the person has used other inappropriate compensatory behavior but has not regularly engaged in self-induced vomiting or misused laxatives, diuretics, or enemas.

Source: American Psychiatric Association, 1994, *Diagnostic and Statistical Manual of Mental Disorders,* 4th ed., Washington, DC: American Psychiatric Association.

displaying emotions, and experience difficulty forming close relationships with others (Fairburn and Brownell 2001). In addition, clinical eating disorders are often accompanied by comorbid psychological conditions, such as obsessive-compulsive disorder, depression, and anxiety disorder (Fairburn and Brownell 2001). For a more comprehensive review of eating disorders in female athletes, see Beals (2004).

The term *subclinical eating disorder* is frequently used to describe athletes who suffer from considerable eating pathology and body weight concerns but do not demonstrate significant psychopathology or fail to meet all of the *DSM-IV* criteria

for anorexia nervosa, bulimia nervosa, or eating disorders not otherwise specified (Beals and Manore 2000; Beals 2004). Many athletes who report using pathogenic weight control methods (e.g., laxatives, diet pills, and excessive exercise) do not technically meet the criteria for a clinical eating disorder (Beals and Manore 2000). The weight control practices utilized by athletes suffering from clinical and subclinical eating disorders are generally severe and extreme (e.g., severe energy restriction or purging) and thus can and often do lead to low EA (Nattiv et al. 2007)

Intentional and rational but mismanaged efforts to reduce body weight can also place a female athlete in a state of low EA. Female athletes, like their nonathletic counterparts, are often concerned with their body weight and shape, and many report actively trying to reduce body weight (Sundgot-Borgen 2002; Beals 2004; Nattiv et al. 2007; Martinsen et al. 2010). For some athletes, weight loss is viewed as a way to improve performance. This is particularly true for athletes who compete in what have been referred to as "thin-build" sports, including endurance, aesthetic, and weight-class sports (Beals 2004). For athletes competing in endurance sports (e.g., distance running, cycling, triathlons, Nordic skiing), a lower body weight is thought to aid in the speed and efficiency of movement and a greater power-to-weight ratio. Athletes competing in aesthetic sports (e.g., gymnastics, diving, figure skating, ballet) often feel pressure to conform to the low body weight and small body size ideals that are typically endorsed by the judges. Finally, in sports that incorporate weight classifications, such as wrestling, rowing, powerlifting, bodybuilding, and karate, athletes often attempt to compete in a lower weight class to improve their chances of winning. It should be noted that performance enhancement is not the only reason female athletes try to lose weight. In fact, research suggests that as many, if not more, female athletes report dieting for "appearance" reasons (Martinsen et al. 2010). Regardless of the reasons for weight loss, reducing energy intake, particularly if the reduction is severe enough, will likely result in low EA.

Inadvertent failure to match energy intake to exercise energy expenditure is believed to occur when the athlete unintentionally consumes inadequate energy to cover her exercise energy expenditure. This unintentional inadequate calorie compensation may be due to time constraints**,** food availability issues, or lack of appropriate nutrition knowledge (Nattiv et al. 2007). It has also been hypothesized that the unconscious failure to increase energy intake in order to match exercise energy expenditure may be due to underlying biological mechanisms. Some researchers contend that "there is no strong biological drive to match energy intake to activity-induced energy expenditure" (Truswell 2001; Loucks 2000, p. 351). Whereas food deprivation generally increases hunger, the same energy deficit produced by exercise training may not (Loucks 2007).

There is some evidence to support the notion that exercise, particularly of an intense and prolonged nature (i.e., endurance exercise) blunts appetite (Borer 2010; King et al. 2010; Stensel 2010; Deighton et al. 2012). For example, King et. al. (2010) examined the effects of prolonged treadmill running on appetite, energy intake, and acylated ghrelin (an appetite-stimulating hormone) in nine healthy males over the course of 24 hours on two occasions (exercise and control) in a randomized-crossover fashion. In the exercise, trial subjects ran for 90 minutes at approximately 70% of Vo_{2max}. Appetite was measured with visual analogue scales and energy intake was assessed from *ad libitum* buffet meals. The results indicated that exercise transiently

suppressed appetite and acylated ghrelin. Furthermore, subjects did not increase energy intake to cover the calories expended in exercise (approximately 5324 kJ); thus, subjects were in a state of inadequate EA on the exercise days. Using a randomized, crossover design, Stubbs et al. (2002) assessed the effects of graded increases in exercise-induced energy expenditure on appetite, daily energy intake, total daily energy expenditure, and body weight in six lean women. The subjects were studied over three separate, 7-day treatments including a no-exercise treatment, a medium exercise treatment (approximately 1.9 MJ/454 kcal/d), and a high exercise treatment (approximately 3.4 MJ/d /812 kcal/d). During all treatments, subjects were allowed to eat *ad libitum*. The results indicated that the subjects only partially compensated for the increase in energy expenditure and the compensation appeared to be less well-matched on the higher energy expenditure day. Unfortunately, similar research has not been conducted on female athletes, so it is unknown whether these results can generalize to that population. Nonetheless, anecdotal reports from female athletes would seem to support the notion that intense and prolonged exercise does blunt appetite and can result in reduced energy intake and a state of low EA.

Research also suggests that the effects of exercise on energy intake may be more extreme during exercise training on a low-fat, high-carbohydrate diet, which is the recommended and typical dietary intake regimen for most endurance athletes. For example, *ad libitum* energy intake did not increase enough to compensate for exercise energy expenditure (approximately 840 kcal/day via cycling) when lean men ate a low-fat, high-carbohydrate diet for 7 days. The effects were additive so that their energy availability exercising on the low-fat, high-carbohydrate diet was only 21 kcal·kg^{-1} FFM·day^{-1} (Stubbs et al. 2004). Similarly, female distance runners (averaging approximately 42 miles/week) consumed approximately 60% of their EEE when provided an *ad libitum* low-fat, high-carbohydrate diet for 28 to 31 days (Horvath et al. 2000).

The mechanisms by which exercise suppresses appetite and leads to inadequate or incomplete compensation in energy intake are not fully understood but may involve lowered concentrations of the appetite hormone, ghrelin, and increased concentrations of satiety hormones, notably peptide YY and glucagon-like peptide 1 (Stensel 2010). Regardless of the mechanism, though, the lack of a full compensatory response of appetite to exercise may facilitate low EA, although there is likely some individual variability in the biological responses and adaptations to exercise (Stensel 2010).

The relative percentage of female athletes with low EA originating from the three underlying causes described above are currently unknown. Such data will likely be difficult to determine not only because there is likely significant overlap between the causes, but due to the methodological difficulties inherent in such exploratory research.

PREVALENCE OF LOW ENERGY AVAILABILITY AMONG FEMALE ATHLETES

The prevalence of low EA with or without disordered eating or eating disorders is currently unknown, largely due to a lack of studies specifically designed to examine prevalence as well as discrepancies in the definition of "low" EA. For example, in one of the first studies to examine EA as part of the newly defined Triad, Hoch et al. (2009) assessed the prevalence of the components of the Triad among female high

school varsity athletes (n = 80) compared to age-matched nonathletic high school girls (n = 80). In this study, low EA was defined as an EA <45 kcal·kg^{-1} FFM·day^{-1} and was found to be present in a similar percentage of athletes (36%) and nonathletes (39%). Nonetheless, more athletes (n = 5) versus nonathletes (n = 3) had EAs less than 30 kcal/kg/FFM. In a similarly designed study, Garneau-Fournier et al. (2011) compared EA between adult (mean age approximately 30 years) female endurance athletes (n = 26) and age-matched, normally active women (n = 12). In this study, low EA was defined as <30 kcal/kg/FFM. Despite the difference in EA definitions, the results were similar to those of Hoch et al. (2009) described above, namely that mean EA was similar between the endurance athletes and the normally active women (37.2 ± 19.3 kcal·kg^{-1} FFM·day^{-1} versus 40.5 ± 14.3 kcal·kg^{-1} FFM·day^{-1}, respectively) and more athletes (n = 7) than control subjects (n = 4) had low EA.

In an attempt to better characterize the range of EAs among female athletes, Beals et al. (2012) examined EA and the prevalence of low EA (defined as an EA ≤30 kcal·kg^{-1} FFM·day^{-1}) among female endurance athletes (n = 40 triathletes, distance runners, and cyclists) training ≥8 hours per week. Assessment of EA was done using 3-day weighed food and activity records designed to capture three distinct training days (heavy, moderate, and easy). The mean EA was 27.8 ± 9.9 kcal·kg^{-1} FFM·day^{-1}, and the majority of athletes (n = 26 or 62.5%) had low EA (≤30 kcal·kg^{-1} FFM·day^{-1}), while only two athletes had an EA that would be considered optimal (≥45 kcal·kg^{-1} FFM·day^{-1}) by current standards.

In the only study to date to examine EA in athletes engaged in an aesthetic sport, Hoch et al. (2011b) assessed the prevalence of "low" EA among professional ballet dancers (n = 22). Energy availability was calculated from the subjects' caloric intake minus the amount of exercise energy expended over the same time as determined from the accelerometer. Low EA was defined as a negative value and using this definition it was determined that 17/22 dancers had low or negative EA.

Based on the limited *direct* evidence described, it would appear that low EA is common among female athletes engaged in endurance and aesthetic sports; however, caution should be exercised when interpreting these results as the number of studies are few, the sample sizes studied were small, and the definitions used for low EA were variable.

Indirect evidence also highlights the prevalence of low EA among female athletes. In a 2007 review article discussing the nutritional aspects of the Triad, Manore et al. (2007) estimated the average EAs from seven published studies that assessed energy intake and exercise energy expenditure among female athletes, most of whom would be classified as endurance athletes (i.e., runners, triathletes, and cyclists). With only one exception, mean EAs were all below 45 kcal·kg^{-1} FFM·day^{-1} with most ≤35 kcal·kg^{-1} FFM·day^{-1}. Mean EAs for amenorrheic athletes were consistently lower than those for eumenorrheic athletes (23 kcal·kg^{-1} FFM·day^{-1} versus 33 kcal·kg^{-1} FFM·day^{-1}, respectively). The mean EAs for the amenorrheic athletes were always ≤30 kcal/kg/FFM, while the mean EAs for the eumenorrhic athletes were significantly more variable, ranging from 19 kcal·kg^{-1} FFM·day^{-1} to 59 kcal·kg^{-1} FFM·day^{-1}.

Taken together, the existing research suggests that low EA is common among female endurance athletes and perhaps those involved in aesthetic sports. However, little is known about the EAs of female athletes participating in other sports.

TABLE 9.2
Reported Energy Availabilities of Female Athletes

Reference	Athlete Population	FFM (kg)	EI (kcal/day)	EEE (kcal/day)	EA (kcal·kg^{-1} FFM·day^{-1})
Beals et al. 2012	Endurance athletes	49	2420	805	28
Garneau-Fournier et al. 2011	Endurance athletes (n = 26)	44	2378	841	35
Tomten and Hostmark 2006	Runners				
	Eumenorrheic (n = 10)	41.1	2940	502	59
	Irregular cycles (n = 10)	44.8	2318	526	40
Thong et al. 2000	Runners and cyclists				
	Eumenorrheic (n = 8)	44.8	2277	956	30
	Amenorrheic (n = 5)	44.8	1672	970	16
Kopp-Woodroffe et al. 1999	Runners and cyclists	50.4	1892	645	25
Laughlin and Yen 1996	Runners and triathletes				
	Eumenorrheic (n = 8)	48.9	1739	906	19
	Amenorrheic (n = 8)	46.4	2106	1074	23
Wilmore et al. 1992	Runners				
	Eumenorrheic (n = 5)	46.6	1690	402	28
	Amenorrheic (n = 8)	45.8	1781	476	28
Myerson et al. 1991	Runners				
	Eumenorrheic (n = 10)	42.9	1934	537	33
	Amenorrheic (n = 7)	43.8	1739	526	28

Source: Adapted from Manore et al. 2007.
Notes: EI, Mean energy intake; EEE, mean exercise energy expenditure; EA, mean energy availability.

Moreover, none of the studies described above attempted to tease out the causes of the low EA among the athletes (i.e., disordered eating versus rational but mismanaged weight loss versus inadvertent failure to meet energy requirements). Nonetheless, as previously mentioned, endurance exercise has been shown to blunt appetite and can induce a state of low EA, at least over the short term (Loucks 2007). Thus, this could explain the low EA, particularly in those studies that examined female endurance athletes, which represents the majority of the research that has been conducted in this area (Table 9.2). On the other hand, female endurance athletes are at an increased risk for disordered eating, which would also induce a state of low EA (Sundgot-Borgen 1993; Beals and Manore 2002; Beals 2004; Sundgot-Borgen and Torstveit 2004; Beals and Hill 2006).

Current estimates of the prevalence of disordered eating among female athletes, including pathogenic weight control behaviors and subclinical and clinical eating disorders, range from less than 1% to as high as 62% in female athletes (Beals 2004). These wide-ranging estimates are due to differences in screening instruments and assessment tools (e.g., self-report questionnaires versus in-depth interviews), definitions of "eating disorders" employed (e.g., few have used the *DSM-IV* criteria), and athletic populations studied (e.g., collegiate versus high school athletes, elite athletes versus recreational

athletes versus physically active people). In fact, to date only five studies have utilized large ($N > 400$), heterogeneous samples of athletes and employed validated measures of disordered eating (Sundgot-Borgen 1993; Johnson et al. 1999; Beals and Manore 2002; Sundgot-Borgen and Torstveit 2004; Torstveit et al. 2008) (Table 9.3). The remainder employed relatively small sample sizes, examined single sports, or used inappropriate measures of disordered eating, all of which can bias prevalence estimates.

As highlighted in Table 9.3, the currently existing research suggests that the prevalence of disordered eating is higher in sports that emphasize a lean physique or a low body weight (i.e., "thin- or lean-build" sports) (Sundgot-Borgen 1993; Beals and Manore 2002; Sundgot-Borgen and Torstveit 2004; Beals and Hill 2006; Tortsveit et al. 2008). It has been hypothesized that the body weight demands of these sports, and the pressure to achieve an ideal body weight, whether real or perceived, cause the female athlete to become overly concerned with her body weight and develop disordered eating behaviors (Sundgot-Borgen 1993; Beals 2004; Torstveit et al. 2008).

Few studies examining disordered eating among athletes assessed energy intake, and none have calculated EA; thus, it is difficult to draw conclusions from the existing data regarding EA in athletes suffering from disordered eating. Nonetheless, it can be assumed that disordered eating behaviors would result in significant reductions in energy intake and thus would lead to a state of low EA. Theoretically then, existing prevalence estimates for disordered eating could be generalized to prevalence estimates of low EA among female athletes. However, more research is needed to confirm this hypothesis.

CONSEQUENCES OF LOW ENERGY AVAILABILITY

Long periods of low EA, with or without disordered eating, can impair the health and physical performance of the female athlete, although the effects appear to be surprisingly variable (Beals 2004; Nattiv et al. 2007). Health consequences of low EA may involve the cardiovascular, reproductive, endocrine, gastrointestinal, renal, and central nervous systems (Manore et al. 2007; Nattiv et al. 2007). In addition, low EA can impair cognitive function and decrease the body's ability to build bone, maintain muscle mass, repair damaged tissue, and recover from injury (Manore et al. 2007).

As previously described, low EA has been shown to suppress reproductive function and reduce markers of bone formation. Assuming that these acute changes in bone translate to long-term changes in BMD, chronically low EA could lead to low BMD and increased risk of stress fractures in female athletes. To date, only one study has directly examined the associations between low EA, BMD, and stress fracture incidence. Beals et al. (2012) examined the prevalence of and associations between low EA, menstrual dysfunction, BMD, and stress fracture incidence among female endurance athletes (n = 40; mean age 27 ± 5 years) training ≥8 hours per week. The results indicated that 62.5% of the athletes had low EA (≤30 kcal·kg^{-1} FFM·day^{-1}); 58% reported a history of amenorrhea and 50% reported currently experiencing irregular menstrual cycles (i.e., cycles not occurring every 28 to 34 days). Eleven percent of the athletes had low spinal BMD (z score ≤–1.0) and 12% (n = 9) reported at least one stress fracture. There were no associations between low

TABLE 9.3
Summary of Prevalence Studies Including Large, Heterogenous Samples of Athletes and Validated Assessments of Disordered Eating

Study	Subjects	Instrument	Findings
Beals and Manore 2002	425 female collegiate athletes	EAT-26 and EDI-BD	3.3% and 2.4% of the athletes self-reported a diagnosis of clinical anorexia and bulimia nervosa, respectively; 15% and 31.5% of the athletes scored above the designated cutoff scores on the EAT-26 and EDI-BD, respectively.
Johnson et al. 1999	1445 collegiate athletes (562 females) from 11 NCAA Division I schools	EDI-2 and questionnaire developed by the authors using *DSM-IV* criteria	1.1% met the criteria for bulimia nervosa, 9.2% met the criteria for subclinical bulimia; 2.8% met the criteria for subclinical anorexia. 5.5% reported purging (vomiting, using laxatives or diuretics) on a weekly basis.
Nichols, Rauh, et al. 2007	423 high school athletes	The Eating Disorder Examination Questionnaire (EDE-Q)	20% met the criteria for DE. No differences in DE prevalence between lean-build and non-lean-build sports.
Sundgot-Borgen 1993	522 Norwegian elite female athletes	EDI and in-depth interview developed by the author based on *DSM III* criteria	1.3%, 8.0%, and 8.2% were diagnosed with anorexia nervosa, bulimia nervosa, and anorexia athletica, respectively.
Sundgot-Borgen and Torstveit 2004	660 Norwegian elite female athletes	A two-stage screening process including a questionnaire developed by the authors, including subscales of the EDI, weight history, and self-reported history of eating disorders (stage 1) followed by a clinical interview using the EDE (stage 2).	21% (n = 121) of the athletes were classified "at risk" after the initial screening. Results of the clinical interview indicated that 2% met the criteria for anorexia nervosa, 6% for bulimia nervosa, 8% for eating disorders not otherwise specified (EDNOS), and 4% for anorexia athletica.
Torstveit et al. 2008	669 Norwegian elite female athletes	A two-stage screening process including a questionnaire developed by the authors, including subscales of the EDI, weight history, and self-reported history of eating disorders (stage 1) followed by a clinical interview using the EDE (stage 2).	23.7% (n = 158) of the athletes reported DE behaviors (stage 1) and were also diagnosed with clinical EDs (stage 2).

Notes: EAT-26, Eating Attitudes Test-26 (Garner et al. 1987); EDI, Eating Disorder Inventory; EDI-BD, body dissatisfaction subscale of the EDI (Garner et al. 1983); EDI-2, Eating Disorder Inventory 2 (Garner 1991); EDE, Eating Disorder Examination (Cooper and Fairburn 1997).

EA and history of amenorrhea, menstrual irregularity, or stress fracture incidence. The lack of associations may have been due to the lack of heterogeneity of EA in the sample (i.e., the high prevalence of low EA among these particular athletes). Additional studies *directly* examining the associations between low EA, BMD, and musculoskeletal injuries are warranted.

Indirect research consistently shows that female athletes suffering from disordered eating are at an increased risk for low BMD and musculoskeletal injuries (Drinkwater et al. 1984; Marcus et al. 1985; Myburgh et al. 1993; Barrack et al. 2008; Koenig et al. 2008; Thein-Nissenbaum et al. 2011; Rauh et al. 2010). Rauh et al. (2010) examined the relationship among disordered eating, menstrual dysfunction, and low BMD and musculoskeletal injury among female high school athletes ($n = 163$). They found that disordered eating and menstrual dysfunction were related to low BMD, and all three were associated with an increased risk of musculoskeletal injuries. In a similar study, Thein-Nissenbaum et al. (2011) found that female high school athletes reporting DE were over two times more likely to sustain a sports-related injury during the competitive season studied. Koenig et al. (2008) examined the case histories of female athletes ($n = 20$) who had suffered stress fractures and stress reactions in the femur and found that several of the athletes reported histories of disordered eating. Finally, Barrack et al. (2008) found that runners who scored high on "dietary restraint" (a common characteristic of those with disordered eating) had lower lumbar spine BMD, bone mineral content, and BMD z-scores.

Research consistently shows that female amenorrheic athletes have lower BMD than eumenorrheic athletes and nonathletic controls, increasing their risk of stress fractures (Rencken et al. 1996; Nichols, Sandborn, et al. 2007; Russell et al. 2009). Because the existing data also indicate that amenorrheic athletes often demonstrate low EA (Manore et al. 2007), it can be assumed that low EA is at least associated with if not a causative factor in low BMD and increased stress fracture risk.

Several investigators have reported a correlation between amenorrhea and endothelial dysfunction in female athletes. This finding is concerning because it is now well accepted that the sentinel event in the development of cardiovascular disease is impaired endothelial function (Rickenlund et al. 2005; Hoch et al. 2003, 2011a, 2011b). Rickenlund et al. (2005) evaluated endothelial function (measured as flow-mediated dilation [FMD] of the brachial artery) and blood markers of cardiovascular disease in female endurance athletes classified by menstrual function status (i.e., amenorrheic, oligomenorrheic, and eumenorrheic) compared to age-matched sedentary controls. There was a direct relationship between the impairment in FMD and the degree of menstrual dysfunction, and the amenorrheic athletes showed significantly lower FMD compared to all other groups (including the sedentary controls). Amenorrheic athletes also had the most unfavorable lipid profile with significantly higher total cholesterol and low-density lipoprotein compared with the other athlete groups. In a similar study, Hoch et al. (2003) found that brachial artery vasodilator response to hyperemia was significantly lower among amenorrheic female distance runners compared to their oligomenorrheic and eumenorrheic counterparts. Neither of these studies examined EA in the athletes; however, as previously described, female distance runners, as an athletic group, are at risk for low EA. In addition, as shown by Manore et al. (2007), low EA is common among amenorrheic athletes.

To date, only one study has attempted to correlate low EA with endothelial dysfunction in female athletes (Hoch et al. 2011b). Hoch et al. (2011b) examined the presence of endothelial function (measured by brachial artery FMD) and its relationship to the components of the Triad among professional ballet dancers (n = 22). The results indicated that 64% of the dancers had impaired brachial artery FMD and that endothelial dysfunction was significantly correlated with menstrual dysfunction and low BMD, but not with low EA. It should be noted, however, that low EA in this study was not commensurate with the current definition of "low EA" (i.e., <30 kcal/kg/FFM^{-1}); rather, it was defined simply as a "negative value" after subtracting exercise energy expenditure from energy intake.

Both observational and experimental data indicate that inadequate energy intake can suppress immune function, especially Type 1 immunity, which is particularly important for protecting against viral pathogens (Calder and Jackson 2000; Loucks et al. 2011). Upper respiratory tract infections, which are caused by viruses, are frequently reported by endurance athletes during intense and prolonged training and competition, and research suggests that it is not the exercise per se, but rather energy and nutrient inadequacies in the face of intense and prolonged exercise that may be the key causative factor (Gleesen et al. 2004; Lancaster et al. 2005; Loucks et al. 2011). For example, Shimizu et al. (2011) found that 2 weeks of weight loss before a competition can impair cell-mediated immune function and induce high susceptibility to upper respiratory tract infections (URTIs) in judo athletes. Similarly, Umeda et al. (2004) documented significant decreases in a number of immunoglobulins among a group of judoists engaging in dietary restriction for weight reduction which were not seen in their weight-stable counterparts. A large survey of Swedish athletes (n = 125 men and 98 women) who had participated in the 2002 and 2004 Olympic games showed that those athletes participating in sports that emphasized leanness reported significantly more attempts to lose weight and significantly more illnesses than athletes participating in sports that did not emphasize leanness.

When EA is low, macronutrient and micronutrient intakes are also likely to be low (Beals and Manore 1998; Manore 1999, 2002). Of particular concern to the female endurance athlete is carbohydrate. Loucks and Thuma (2003) found that low energy availability, caused by dietary energy restriction, greatly reduced carbohydrate intake. Consequently, skeletal muscle derived much less energy from carbohydrate oxidation in the state of deprived energy availability compared to adequate energy availability. Performance in a high-intensity endurance activity fueled primarily by glucose metabolism will be compromised by low carbohydrate intake. Moreover, research has consistently shown that exercising in a carbohydrate-depleted state suppresses immune response (Mitchel et al. 1998; Gleeson et al. 2004; Lancaster et al. 2005). For example, Lancaster et al. (2005) examined the effects of carbohydrate ingestion on immune response in male endurance athletes (n = 7) undergoing a 2.5-hour cycling bout at 65% VO_{2max}. The results indicated that compared to a placebo condition, the ingestion of carbohydrate reduced the suppression of Type 1 immune defenses by an average of 65%. Similarly, Mitchell et al. (1998) observed that exercising (1 hour at 75% VO_{2max}) in a glycogen-depleted state (brought about by 2 days on a low-carbohydrate diet) resulted in a greater fall in circulating lymphocyte numbers 2 hours after exercise compared with the same exercise performed after 2 days

on a high-carbohydrate diet in a group of healthy males. Unfortunately, there are no similar studies examining the effects of carbohydrate ingestion on immune function in female athletes. Given the differences in substrate utilization, particularly carbohydrate oxidation, between males and females at submax exercise intensities, generalizations of the findings in male to female athletes should be considered with caution.

Female athletes who consume insufficient calories are also likely not getting enough protein to synthesize and maintain lean body mass and to replace any protein used for energy during exercise (Beals and Manore 1998; Beals 2004). While protein typically contributes 10% or less of the fuel used during exercise, the amount may be considerably larger if carbohydrate (i.e., glucose) is in short supply or if energy intake is low (i.e., during periods of dieting). Without enough protein to maintain and repair muscle tissue, the athlete increases her risk of muscle wasting, weakness, and injury.

Female athletes with inadequate energy intake are also likely to have poor vitamin and mineral intakes. This is particularly true for the B-complex vitamins (thiamin, riboflavin, niacin, vitamin B_6, folate, and vitamin B_{12}) (Woolf and Manore 2006) and the minerals iron, calcium, zinc, and magnesium (Manore 1999, 2002). These micronutrients are especially important for the athlete because they play central roles in energy production, hemoglobin synthesis, muscle tissue synthesis and repair, bone health, and immune function. Poor micronutrient status thus can lead to fatigue, muscle weakness, musculoskeletal injuries, and an increased risk of infections (Manore 1999, 2002).

STRATEGIES TO CORRECT LOW ENERGY AVAILABILITY AMONG FEMALE ATHLETES

Ideally, correction of low EA should focus on increasing energy intake while also reducing exercise EE without negatively impacting the athlete's training regimen and potentially hindering performance. To date, there are no studies that have examined the impact of the restoration or normalization of EA on both the health *and* performance of the female athlete. However, there are a few intervention studies that have documented the effects of increasing energy intake and decreasing exercise energy expenditure on various aspects of health (e.g., menstrual function, markers of bone turnover, bone mineral density) of the female athlete. These will be described briefly below.

Using a case-study approach, Dueck et al. (1996) examined the effects of a 15-week diet and exercise intervention program on energy balance, hormonal profiles, body composition, and menstrual function in an amenorrheic distance runner. The intervention consisted of reducing the athlete's training volume (by adding an additional "rest" day) and increasing energy intake by 350 kcal/day, which resulted in increasing EA by 8 (vs. 5) $kcal \cdot kg^{-1}$ $FFM \cdot day^{-1}$. As a result of the intervention, the athlete experienced a transition from negative to positive energy balance, an improvement in hormonal profile, and a resumption of menses. A follow-up study employing an additional four subjects and a slightly longer intervention period (i.e., 20 weeks) found similar positive effects on energy balance, hormonal profile, and menstrual

function with the added benefit of improvements in bone mineral density at the lumbar spine and femoral neck (Kopp-Woodroffe 1999). The athletes in this study also self-reported positive effects on overall energy, concentration, and mood state.

Researchers from Oregon State University recently completed a series of studies (with the acronym REMEDY) designed to examine the effects of improving EA on various health parameters (e.g., menstrual function, BMD, muscle strength, resting metabolic rate) in female, endurance athletes ($n = 8$, 7 amenorrheic and 1 oligomenorrheic) (Ciadella-Kam et al. 2011; Gruebels et al. 2011). The 6-month intervention study required the athletes to consume a liquid carbohydrate/protein supplement that provided an additional 360 kcal/day. Although the authors reported that EA did not change *significantly* over the 6-month intervention period, it should be noted that mean EA did increase from 34 kcal/kg/day to 41 kcal/kg/day, and all athletes resumed menses. Thus, although not statistically significant, the increase in EA clearly had clinical significance from a menstrual status standpoint. No significant changes were seen overall for BMD, although women with the lowest BMD did experience significant improvements. The only lower body strength measurement that was improved was knee extensor strength. The authors speculated that more time may be required after the resumption of menses before improvements in BMD and muscle strength are demonstrated.

While the above data convincingly show that increasing EA can have positive effects on various health parameters and may improve performance measures, convincing a female athlete to consume more calories (with or without a reduction in training) can prove to be very difficult, particularly if the athlete competes in a sport in which body weight or body shape is believed to have an impact on performance. Thus, intervention strategies should aim to employ small, gradual increases in EA while maintaining or slightly decreasing exercise energy expenditure with frequent assessments of the athlete's health and performance to evaluate the impact of the intervention.

The intervention studies cited above all utilized a liquid dietary supplement to increase energy intake. This was likely done for experimental control as well as convenience. Certainly an increase in energy intake could be achieved by adding whole foods to the diet. However, a nutritional supplement (a drink, powder, or bar) may be easier for the athlete to incorporate into her diet and compliance may be better. Such supplements are generally balanced in macronutrient content and provide additional vitamins and minerals that may be beneficial to the female athlete's diet. They are convenient and easy to consume. If left to choose her own foods, the athlete may choose those that provide volume without the necessary calories to boost EA significantly. For example, Reed et al. (2011) found that female athletes with exercise-associated menstrual disturbances maintained a lower-calorie diet than their eumenorrheic counterparts by consuming a lower-energy diet through a higher intake of vegetables, condiments, and noncaloric beverages. The provision of calories in a more condensed form, such as a beverage or bar, may be more tolerable to the female athlete, particularly one who is concerned with how the extra calories may make her feel. Moreover, because liquid calories do not seem to affect satiety to the same degree as solid calories (Apolzan et al. 2011), the athlete may not be tempted to "compensate" for the additional calories by reducing the intake of other foods throughout the day.

A gradual increase in energy intake will give the athlete time to get used to the additional calories (and any weight change that may occur as a result). The intervention studies cited indicated that a side effect of the intervention was weight gain in the athletes (approximately 1 to 2 kg). Although considered relatively small by normal standards, it can be concerning to female athletes, particularly those who participate in endurance or aesthetic sports.

Reducing exercise energy expenditure by decreasing the athlete's training load will likely prove to be more difficult than increasing energy intake, as the athlete may be concerned about how the reduction in training will impact performance. A careful review of the athlete's current training regimen and training history will help to identify opportunities where exercise energy expenditure can be reduced. Specifically, look for any superfluous activity the athlete might be engaging in that appears to be more for sheer energy expenditure than training (i.e., using the elliptical for 20 min after a 2-hour soccer practice), and reduce or eliminate that first. With respect to the training program, a greater focus should be placed on quality versus quantity of training. Reducing longer duration exercise sessions while maintaining the shorter, high-intensity training sessions will help to promote a slight reduction in exercise energy expenditure. Encourage athletes who are not currently taking a "rest" day to incorporate at least one into the training week. This will not only positively affect EA but likely provide a performance benefit as overtraining is well known to negatively affect performance (Purvis et al. 2010).

As previously indicated, female athletes with inadequate energy intakes are also likely to have inadequate micronutrient intakes. Ideally, these nutrient inadequacies should be met by increasing energy intake and with whole foods. Nonetheless, given the necessity of a gradual increase in energy intake along with the reality of the struggle to get some female athletes to "eat more," vitamin and mineral supplements may be warranted, at least until the athlete's EA is adequate and there is evidence of micronutrient sufficiency. Not surprisingly, the micronutrients highlighted in the other chapters found within this book (i.e., B-vitamins, iron, zinc, and the bone nutrients, particularly calcium and vitamin D) are the ones that female athletes, especially those with low EA, should focus on.

A multivitamin mineral supplement containing 100% of the RDA for most vitamins and minerals will meet the needs of most female athletes with low EA. It has been recommended that female athletes with menstrual dysfunction and low BMD consume 1500 mg/day of calcium and at least the RDA for vitamin D (i.e., 600 IU/day or 15 mg/day). Some researchers have suggested that higher intakes of vitamin D (800 to 2000 IU/day) may be beneficial from a bone-health standpoint, and while the hypothesis has not yet been tested in a female athlete population, such levels are still below the current upper intake level (UL) of 4000 I/day and are likely not harmful. As indicated in Chapter 5, if iron status tests are indicative of iron deficiency, then iron supplementation under the supervision of the athlete's physician and a registered dietitian is warranted. Finally, there is some research to suggest that folic acid supplementation may be beneficial for treating the endothelial dysfunction that often accompanies low EA and menstrual dysfunction in female athletes (Hoch et al. 2010, 2011a; Lanser et al. 2011). To date, three studies have demonstrated significant improvements in FMD with folic acid supplementation (10 mg/day for 4 weeks)

in female athletes (runners and ballet dancers) (Hoch et al. 2010, 2011a; Lanser et al. 2011). Nonetheless, given that this dose far exceeds the UL, athletes should not undertake such supplementation without the supervision of a physician or other qualified health professional.

SUMMARY

The limited existing research indicates that low EA is common among female athletes, particularly those engaging in endurance and aesthetic sports (i.e., "thin-build" sports). Risk of low EA seems to be heightened in these athletes due to body weight and shape concerns and resulting weight management practices along with possible effects of intense exercise on appetite and energy intake. Indirect evidence as well as some limited direct evidence suggest that low EA can have profound negative effects on the health, nutritional status, and even performance of the female athlete. Thus, until research indicates otherwise, it would be prudent for health and nutritional professionals to ensure that female athletes achieve an EA that corresponds to normal menstrual function, optimal BMD, and macro- and micronutrient adequacy. Such a value is likely to be >30 kcal·kg^{-1} FFM·day^{-1}; however, how much above this value is currently unknown and will likely be dependent upon a number of factors that are unique to the individual female athlete.

REFERENCES

American Psychiatric Association. 1994. *Diagnostic and statistical manual of mental disorders.* 4th ed. Washington, DC: Author.

Apolzan, J. W., Leidy, H. J., Mattes, R. D., and Campbell, W. W. 2011. Effects of food form on food intake and postprandial appetite sensations, glucose and endocrine responses, and energy expenditure in resistance trained v. sedentary older adults. *Br J Nutr*. 106:1107–1116.

Barrack, M. T., Rauh, M. J., Barkai, H. S., and Nichols, J. F. 2008. Dietary restraint and low bone mass in female adolescent endurance runners. *Am J Clin Nutr*. 87:36–43.

Beals, K. A. 2004. *Disordered Eating Among Athletes: A Comprehensive Guide for Health Professionals*. Champaign, IL: Human Kinetics.

Beals, K. A., Henderson, K., and Dorias, J. 2012. Associations between energy availability, menstrual function and bone health in female endurance athletes. *Med Sci Sports and Exerc*. Abstr.

Beals, K. A., and Hill, A. K. 2006. The prevalence of disordered eating, menstrual dysfunction, and low bone mineral density among U.S. collegiate athletes. *Int J Sport Nutr Exerc Metab.* 16:1–23.

Beals, K. A., and Manore, M. M. 2000. Behavioral, psychological, and physical characteristics of female athletes with subclinical eating disorders. *Int J Sport Nutr Exerc Metab.* 10:128–143.

Beals, K. A., and Manore, M. M. 2002. Disorders of the female athlete triad among collegiate athletes. *Int J Sport Nutr Exerc Metab.* 12:281–293.

Beals, K. A., and Manore, M. M. 1998. *J Am Diet Assoc*. 98:419–425.

Borer, K. T. 2010. Nonhomeostatic control of human appetite and physical activity in regulation of energy balance. *Exer Sport Sci Rev*. 38:114–121.

Budgett, R. 1998. Fatigue and underperformance in athletes: The overtraining syndrome. *Br J Sports Med*. 32:107–110.

Calder, P. C., and Jackson, A. A. 2000. Undernutrition, infection and immune function. *Nutr Res Rev*. 13:3–29.

Ciadella-Kam, L., Guebels, C., Maddalozzo, G., and Manore, M. M. 2011. REMEDY: Impact of menstrual status on musculoskeletal and hormonal health. *Med Sci Sport Exerc*. 43(suppl):S47.

Deighton, K., Zahra, J. C., and Stensel, D. J. 2012. Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state. *Appetite* 58(3):946–954.

De Souza, M. J., Miller, B. E., Loucks, A. B., Luciano, A. A., Pescatello, L. S., Campbell, C. G., et al. 1998. High frequency of luteal phase deficiency and anovulation in recreational women runners: Blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. *J Clin Endocrin Metab.* 83:4220.

Drinkwater, B. L., Nilson, K., Chesnut, C. H. 3rd, Bremner, W. J., Shainholtz, S., Southworth, M. B. 1984. Bone mineral content of amenorrheic and eumenorrheic athletes. *N Engl J Med*. 311:277–281.

Dueck, C. A., Matt, K. S., Manore, M. M., and Skinner, J. S. 1996. Treatment of athletic amenorrhea with a diet and training intervention program. *Int J Sport Nutr*. 6:24–40.

Fairburn, C. G., and Brownell, K. D., eds. 2001. *Eating Disorders and Obesity: A Comprehensive Handbook.* 2nd ed. New York: Guilford Press.

Garneau-Fournier, J., Meyer, N., Subudhi, A., and Berning, J. 2011. Low energy availability and the female athlete triad in endurance athletes versus controls. *Med Sci Sports Exerc.* 43:S47.

Garner, D. M. *Eating disorder inventory-2 manual*. 1991. Odessa, FL: Psychological Assessment Resources.

Garner, D. M., Olmstead, M. P., Bohr, Y., and Garfinkel, P. E. 1982. The Eating Attitudes Test: Psychometric features and clinical correlates. *Psychol Med*. 12:871–878.

Garner, D. M., Olmsted, M. P., and Polivy, J. 1983. Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia. *Int J Eat Disord*. 2:15–34.

Gleeson, M., Nieman, D. C., and Pedersen, B. K. 2004. Exercise, nutrition and immune function. *J Sports Sci*. 22:115–125.

Gruebels, C. P., Cialdella-Kam, L., Maddalozzo, G., and Manore, M. M. 2011. REMEDY: Menstrual status and energy availability in active women. *Med Sci Sport Exerc*. 43(suppl):S47.

Hoch, A. Z., Dempsey, R. L., Carrera, G. F., Wilson, C. R., Chen, E. H., Barnabei, V. M., Sandford, P. R., Ryan, T. A., and Gutterman, D. D. 2003. Is there an association between athletic amenorrhea and endothelial cell dysfunction? *Med Sci Sports Exerc*. 35:377–383.

Hoch, A. Z., Lynch, S. L., Jurva, J. W., Schimke, J. E., and Gutterman, D. D. 2010. Folic acid supplementation improves vascular function in amenorrheic runners. *Clin J Sport Med.* 20:205–210.

Hoch, A. Z., Pajewski, N. M., Moraski, L., Carrera, G. F., Wilson, C. R., Hoffmann, R. G., Schimke, J. E., and Gutterman, D. D. 2009. Prevalence of the female athlete triad in high school athletes and sedentary students. *Clin J Sport Med*. 19:421–428.

Hoch, A. Z., Papanek, P., Szabo, A., Widlansky, M. E., and Gutterman, D. D. 2011a. Folic acid supplementation improves vascular function in professional dancers with endothelial dysfunction. *PM R*. 3:1005–1012.

Hoch, A. Z., Papanek, P., Szabo, A., Widlansky, M. E., Schimke, J. E., and Gutterman, D. D. 2011b. Association between the female athlete triad and endothelial dysfunction in dancers. *Clin J Sport Med*. 21:119–125.

Horvath, P. J., Eagen, C. K., Ryer-Calvin, S. D., and Pendergast, D. R. 2000. The effects of varying dietary fat on the nutrient intake in male and female runners. *J Am Coll Nutr*. 19:42–51.

Ihle, R., and Loucks, A. B. 2004. Dose-response relationships between energy availability and bone turnover in young exercising women. *J Bone Miner Res.* 19:1231–1240.

Johnson, C., Powers, P. S., and Dick, R. W. 1999. Athletes and eating disorders: The national collegiate athletic association study. *Int J Eat Disord.* 26:179–188.

King, J. A., Miyashita, M., Wasse, L. K., and Stensel, D. J. 2010. Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* 54:492–498.

Koenig, S. J, Toth, A. P., and Bosco, J. A. 2008. Stress fractures and stress reactions of the diaphyseal femur in collegiate athletes: An analysis of 25 cases. *Am J Orthop.* 37:476–480.

Kopp-Woodroffe, S. A., Manore, M. M., Dueck, C. A., Skinner, J. S., and Matt, K. S. 1999. Energy and nutrient status of amenorrheic athletes participating in a diet and exercise training intervention program. *Int J Sport Nutr*. 9:70–88.

Lancaster, G. I., Khan, Q., Drysdale, P. T., Wallace, F., Jeukendrup, A. E., Drayson, M. T., and Gleeson, M. 2005. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. *J Appl Physiol*. 98:565–571.

Lanser, E. M., Zach, K. N., and Hoch, A. Z. 2011. The female athlete triad and endothelial dysfunction. *PM R.* 3:458–465.

Laughlin, G. A., and Yen, J. 1996. Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. *Clin Endocrinol Metab.* 81:4301–4309.

Loucks, A. B. 2007. Low energy availability in the marathon and other endurance sports. *Sports Med*. 37:348–352.

Loucks, A. B., Kiens, B., and Wright, H. H. 2011. Energy availability in athletes. *J Sports Sci*. 29(Suppl. 1):S7–S15.

Loucks, A. B., and Thuma, J. R. 2003. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab.* 88:297–311.

Loucks, A. B., Verdun, M., and Heath, E. M. 1998. Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. *J Appl Physiol.* 84:37–46.

Manore, M. M. 1999. Nutritional needs of the female athlete. *Clin Sports Med*. 18:549–563.

Manore, M. M. 2002. Dietary recommendations and athletic menstrual dysfunction. *Sports Med*. 32:887–901.

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

Marcus, R., Cann, C., Madvig, P., Minkoff, J., Goddard, M., Bayer, M., Martin, M., Gaudiani, L., Haskell, W., and Genant, H. 1985. Menstrual function and bone mass in elite women distance runners. Endocrine and metabolic features. *Ann Intern Med*. 102:158–163.

Martinsen, M., Bratland-Sanda, S., Eriksson, A. K., and Sundgot-Borgen, J. 2010. Dieting to win or to be thin? A study of dieting and disordered eating among adolescent elite athletes and non-athlete controls. *Br J Sports Med*. 44:70–76.

Mitchell, J. B., Pizza, F. X., Paquet, A., Davis, B. J., Forrest, M. B., and Braun, W. A. 1998. Influence of carbohydrate status on immune responses before and after endurance exercise. *J Appl Physiol.* 84:1917–1925.

Myburgh, K. H., Bachrach, L. K., Lewis, B., Kent, K., and Marcus, R. 1993. Low bone mineral density at axial and appendicular sites in amenorrheic athletes. *Med Sci Sports Exerc*. 25:1197–1202.

Myerson, M., Gutin, B., Warren, M. P., May, M. T., Contento, I., Lee, M., Pi-Sunyer, F. X., Pierson, R. N. Jr., and Brooks-Gunn, J. 1991. Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. *Med Sci Sports Exerc.* 23:15–22.

Nattiv, A., Loucks, A. B., Manore, M. M., Sanborn, C. F., Sundgot-Borgen, J., and Warren M. P. 2007. American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc.* 39:1867–1882.

Nichols, J. F., Rauh, M. J., Barrack, M. T., Barkai, H. S., and Pernick, Y. 2007. Disordered eating and menstrual irregularity in high school athletes in lean-build and nonlean-build sports**.** *Int J Sport Nutr Exerc Metab*. 17:364–377.

Nichols, D. L., Sanborn, C. F., and Essery, E. V. 2007. Bone density and young athletic women. An update. *Sports Med*. 37:1001–1014.

Pollock, N., Grogan, C., Perry, M., Pedlar, C., Cooke, K., Morrissey, D., and Dimitriou, L. 2010. Bone-mineral density and other features of the female athlete triad in elite endurance runners: A longitudinal and cross-sectional observational study. *Int J Sport Nutr Exerc Metab*. 20:418–426.

Purvis, D., Gonsalves, S., and Deuster, P. A. 2010. Physiological and psychological fatigue in extreme conditions: Overtraining and elite athletes. *PM R*. 2:442–450.

Rauh, M. J., Koepsell, T. D., Rivara, F. P., Margherita, A. J., and Rice, S. G. 2006. Epidemiology of musculoskeletal injuries among high school cross-country runners. *Am J Epidemiol.* 163:151–159.

Rauh, M. J., Nichols, J. F., and Barrack, M. T. 2010. Relationships among injury and disordered eating, menstrual dysfunction, and low bone mineral density in high school athletes: A prospective study. *J Athl Train*. 45:243–252.

Reed, J. L., Bowell, J. L., Hill, B. R., Williams, B. A., DeSouza, M. J., and Williams, N. I. 2011. Exercising women with menstrual disturbances consume low energy dense foods and beverages. *Appl Physiol Nutr Metab*. 36:382–394.

Rencken, M. L., Chesnut, C. H. 3rd, and Drinkwater, B. L. 1996. Bone density at multiple skeletal sites in amenorrheic athletes. *JAMA* 276:238–240.

Rickenlund, A., Eriksson, M. J., Schenck-Gustafsson, K., and Hirschberg, A. L. 2005. Amenorrhea in female athletes is associated with endothelial dysfunction and unfavorable lipid profile. *J Clin Endocrinol Metab*. 90:1354–1359.

Russell, M., Stark, J., Nayak, S., Miller, K. K., Herzog, D. B., Klibanski, A., and Misra, M. 2009. Peptide YY in adolescent athletes with amenorrhea, eumenorrheic athletes and non-athletic controls. *Bone* 45:104–109.

Shimizu, K., Aizawa, K., Suzuki, N., Masuchi, K., Okada, H., Akimoto, T., Mesaki, N., Kono, I., and Akama, T. 2011. Influences of weight loss on monocytes and T-cell subpopulations in male judo athletes. *J Strength Cond Res*. 25:1943–1950.

Stensel, D. 2010. Exercise, appetite and appetite-regulating hormones: Implications for food intake and weight control. *Ann Nutr Metab*. 57 (Suppl 2):36–42.

Stubbs, R. J., Hughes, D. A., and Johnstone, A. M., et al. 2004. Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. *Am J Physiol Regul Integr Comp Physiol.* 286:R350–R358.

Stubbs, R. J., Sepp, A., Hughes, D. A., Johnstone, A. M., King, N., Horgan, G., and Blundell, J. E. 2002. The effect of graded levels of exercise on energy intake and balance in free-living women. *Int J Obes Relat Metab Disord*. 26:866–869.

Sundgot-Borgen, J. 1993. Prevalence of eating disorders in elite female athletes. *Int J Sport Nutr*. 3:29–40.

Sundgot-Borgen, J. 2002. Weight and eating disorders in elite athletes. *Scand J Med Sci Sports*. 12:259–260.

Sundgot-Borgen, J., and Torstveit, M. K. 2004. Prevalence of eating disorders in elite athletes is higher than in the general population. *Clin J Sport Med*. 14:25–32.

Thein-Nissenbaum, J. M., Rauh, M. J., Carr, K. E., Loud, K. J., and McGuine, T. A. 2011. Associations between disordered eating, menstrual dysfunction and musculoskeletal injury in high school athletes. *J Orthop Sports Phys Ther.* 41:60–69.

Thong, F. S., McLean, C., and Graham T. E. 2000. Plasma leptin in female athletes: Relationship with body fat, reproductive, nutritional and endocrine factors. *J Appl Physiol.* 88:2037–2044.

Tomten, S. E., and Hostmark, A. T. 2006. Energy balance in weight stable athletes with and without menstrual disorders. *Scand J Med Sci Sports* 16:127–133.

Torstveit, M. K., Rosenvinge, J. H., and Sundgot-Borgen, J. 2008. Prevalence of eating disorders and the predictive power of risk models in female elite athletes: A controlled study. *Scand J Med Sci Sports*. 18:108–118.

Truswell, A. S. 2001. Energy balance, food and exercise. *World Rev Nutr Diet.* 90:13–25.

Umeda, T., Nakaji, S., Shimoyama, T., Kojima, A., Yamamoto, Y., and Sugawara, K. 2004. Adverse effects of energy restriction on changes in immunoglobulins and complements during weight reduction in judoists. *J Sports Med Phys Fitness*. 44:328–334.

Wade, G. N., and Jones, J. E. 2004. Neuroendocrinology of nutritional infertility. *Am J Physiol, Regul, Integr Comp Physiol*. 287:R1277–R1296.

Willis, K. S., Peterson, N. J., and Larson-Meyer, D. E. 2008. Should we be concerned about the vitamin D status of athletes? *Int J Sport Nutr Exerc Metab.* 18:204–224.

Wilmore, J. H., Wambsgans, K. C., Brenner, M., Broeder, C. E., Paijmans, I., Volpe, J. A., and Wilmore, K. M. 1992. Is there energy conservation in amenorrheic compared with eumenorrheic distance runners? *J Appl Physiol.* 72:15–22.

Winters-Stone, K. M., and Snow, C. M. 2004. One year of oral calcium supplementation maintains cortical bone density in young adult female distance runners. *Int J Sport Nutr Exerc Metab*. 14:7–17.

Woolf, K., and Manore, M. M. 2006. B-vitamins and exercise: Does exercise alter requirements? *Int J Sport Nutr Exerc Metab.* 16:453–484.

Zanker, C. L., and Swaine, I. L. 1998. Bone turnover in amenorrhoeic and eumenorrhoeic women distance runners. *Scand J Med Sci Sports.* 8:20–26.

10 Menstrual Function and Dysfunction in the Female Athlete

Anne B. Loucks

CONTENTS

INTRODUCTION

Menstrual disorders are common among female athletes, especially adolescents, but they should not be accepted as part of the life of an athlete, because they may be a sign of pathology. Many pathologies can cause menstrual disorders. The underlying origin of pathology in the Female Athlete Triad (the *Triad*) is believed to be low

energy availability (i.e., eating too few calories to support basic physiological functions in addition to exercise).* The consequences of these menstrual disorders are many and may include low bone mineral density, poor cold tolerance, abnormally short or tall stature depending on the age at which energy deficiency occurs, reduced endurance, and so forth. Because effects on bone may be irreversible, early detection and prompt treatment of menstrual dysfunction are imperative. This can be difficult, however, because some menstrual disorders have no overt signs or symptoms. This chapter clarifies the difference between menstrual irregularity and menstrual disorders and comments on the prevalence of menstrual disorders among female athletes. It then presents evidence suggesting that low energy availability is the underlying cause of menstrual disorders in the Triad and explains their treatment options.

REGULATION OF THE FEMALE REPRODUCTIVE SYSTEM

In women, the secretory organs of the reproductive axis include the hypothalamus, pituitary gland, and ovary. Thus, the axis is often referred to as the hypothalamic-pituitary-ovarian (HPO) axis. The glands of the HPO axis secrete hormones rhythmically. Pulses of gonadotropin-releasing hormone (GnRH) secreted by specialized neurons in the hypothalamus are carried in a network of portal veins to the nearby pituitary gland where they stimulate gonadotroph cells to secrete pulses of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the general circulation. Pulses of LH are detectable in the blood, and the fertility of a woman depends upon the frequency of these pulses.

The LH pulse pattern varies during the menstrual cycle, as the release of GnRH and LH are modulated by the feedback of estradiol and progesterone secretions from the ovary, which also vary during the cycle. In an eumenorrheic, cyclic sedentary (CS) young woman, the 24-hour LH profile in the early follicular phase, when circulating estrogen and progesterone levels are low, is characterized by regular, high-frequency (~1/h), low-amplitude pulses (Figure 10.1) (CS) (Loucks et al. 1989). During sleep, the frequency slows and the amplitude increases.

In response to the proper pulsatile and monthly rhythmic stimulation by LH and FSH, clusters of ovarian cells (*ovarian follicles*) grow and secrete increasing amounts of estrogen (Figure 10.2) (CS). Gradually, one (on average) of the follicles becomes dominant while others diminish. Eventually the rising concentration of estrogen secreted into the blood by the dominant follicle exerts a positive feedback on LH. The resulting spike in the concentration of LH causes the dominant follicle to rupture, thereby releasing an egg cell for fertilization. The remaining cells of the dominant follicle then undergo rapid chemical and morphological changes to form the *corpus luteum*, a body that begins secreting progesterone as well as estrogen into the blood. The interval during which a dominant follicle develops, from menses until ovulation, is known as the *follicular phase* of the menstrual cycle. The interval

* Information about other pathological causes of menstrual disorders is available elsewhere—see Speroff, L., and M. A. Fritz (2005), *Clinical Gynecologic Endocrinology and Infertility*, Philadelphia: Lippincott Williams & Wilkins.

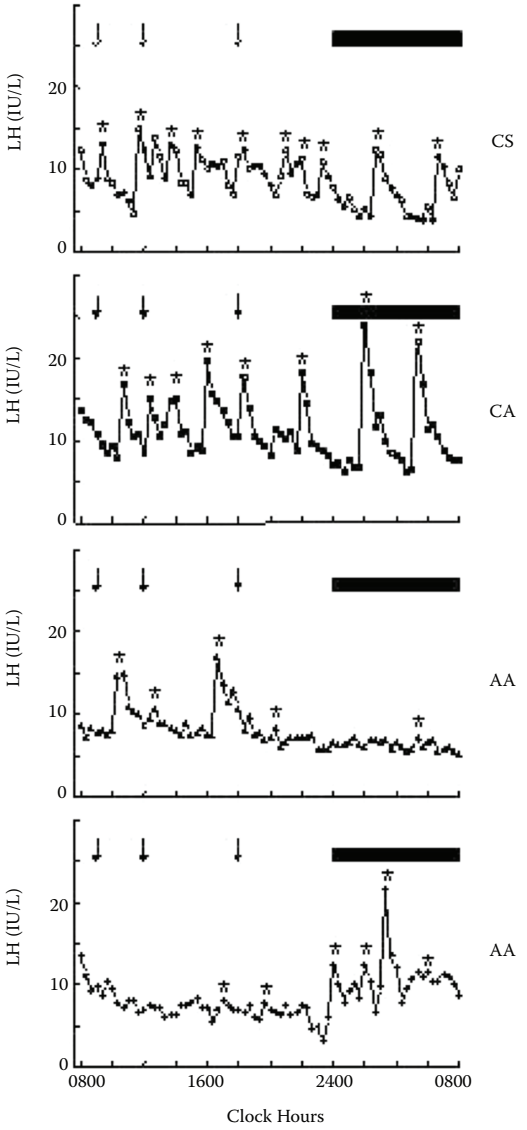

FIGURE 10.1 The 24-hour rhythm of serum luteinizing hormone (LH) levels at 20-min intervals in a typical cyclic (i.e., eumenorrheic) sedentary (CS) woman, a typical cyclic athlete (CA), and two amenorrheic athletes (AAs). Asterisks indicate pulses detected by a computer program using objective pulse detection criteria. Arrows indicate meal times. Horizontal bars indicate sleep periods. The effects of LH on ovarian function were determined by the frequency of LH pulses, not the average LH concentration. (Loucks, A. B., Mortola, J. F., Girton, L., and Yen, S. S. C., 1989, Alterations in the Hypothalamic–Pituitary–Ovarian and the Hypothalamic–Pituitary–Adrenal Axes in Athletic Women, *J Clin Endocrinol Metab*, 68, 402–411. With permission.)

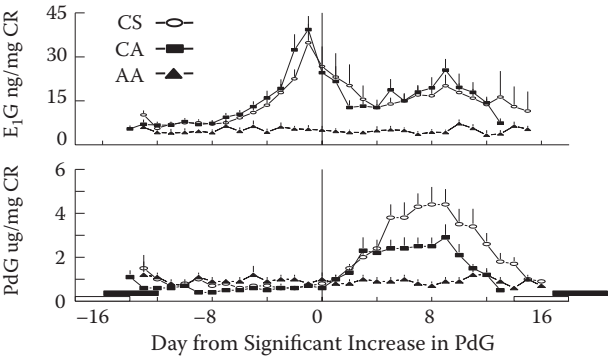

FIGURE 10.2 Mean (+SE) daily urinary excretion of the estrogen metabolite estrone glucuronide (E_1G) (*top*) and the progesterone metabolite pregnanediol glucuronide (PdG) (*bottom*) in cyclic (i.e., eumenorrheic) sedentary (CS) women, cyclic athletes (CAs), and amenorrheic athletes (AAs). Data are presented over one menstrual cycle for cyclic women and over 30 arbitrary days for amenorrheic women. Days are oriented from a significant increase in urinary PdG excretion, with day 1 being the day of the first significant increase. Filled bar, days of menses in CS; open bar, days of menses in CA. AAs display no evidence of follicular development, ovulation, or luteal function. Luteal function is suppressed and abbreviated in CAs. (Loucks, A. B., Mortola, J. F., Girton, L., and Yen, S. S. C., 1989, Alterations in the Hypothalamic–Pituitary–Ovarian and the Hypothalamic–Pituitary–Adrenal Axes in Athletic Women, *J Clin Endocrinol Metab*, 68, 402–411. With permission.)

during which the corpus luteum is active, from ovulation until the next menses, is known as the *luteal phase*.

Estrogen and progesterone have profound influences on the uterine endometrium and on other tissues including bone, the breast, and the vascular endothelium. Estrogen stimulates endometrial proliferation, and progesterone causes it to become highly vascularized. These changes create hospitable conditions for the implantation of a fertilized egg into the endometrium. If no fertilization occurs within several days, the ability of the corpus luteum to secrete progesterone becomes exhausted, the structural integrity of the endometrium collapses, and menstruation begins. Usually the secretory capacity of the corpus luteum is sustained long enough for the rapidly dividing cells derived from a fertilized egg to become implanted in the endometrium 6 or 7 days after fertilization. If the secretory capacity of the corpus luteum is exhausted too soon, the endometrium sloughs off before implantation can occur. The likelihood of this happening increases when the luteal phase is shorter than 10 days.

In *eumenorrhea*, menstrual cycles occur at intervals of 25 to 35 days. Such regular intervals are usually ovulatory, but not always. In *oligomenorrhea*, cycles occur at intervals longer than 35 days, and at less regular intervals than in eumenorrhea. In *amenorrhea*, the absence of menses usually implies anovulation with no cyclic variation of estradiol and progesterone concentrations. Thus, on any given day, hormone concentrations and responses of the HPO axis to stimulation are distinctly and predictably different in amenorrheic and eumenorrheic women. By contrast, in oligomenorrhea, hormone concentrations and the responsiveness of the HPO axis

are difficult to predict, because cycles occur at irregular intervals and the lengths of their follicular and luteal phases are also irregular.

Many investigators have mistakenly combined amenorrheic and oligomenorrheic athletes into a single "oligo/amenorrheic" group, because they all have menstrual disorders. Except for the very first few days of each cycle, however, hormone concentrations and HPO axis responsiveness of oligomenorrheic women are unlike those of amenorrheic women. On most days, they are like those of eumenorrheic women, although not on the same days. Therefore, it is never appropriate to combine oligomenorrheic and amenorrheic women into an oligo/amenorrheic group for assessing the integrity of the reproductive system, or any other system, such as the skeletal system, that is affected by the reproductive system.

THE MYTH OF THE "NORMAL" MENSTRUAL CYCLE

When asked how often their menstrual cycles occur, most women answer "every 28 days" (Treloar et al. 1967), but when they record their menstrual cycles prospectively on a calendar, analysis of the calendars reveals that only 13% of menstrual cycles are actually 28 days long (Figure 10.3). Insofar as menstrual length is concerned, "each woman has her own central trend and variation, both of which change with age" (Treloar et al. 1967).

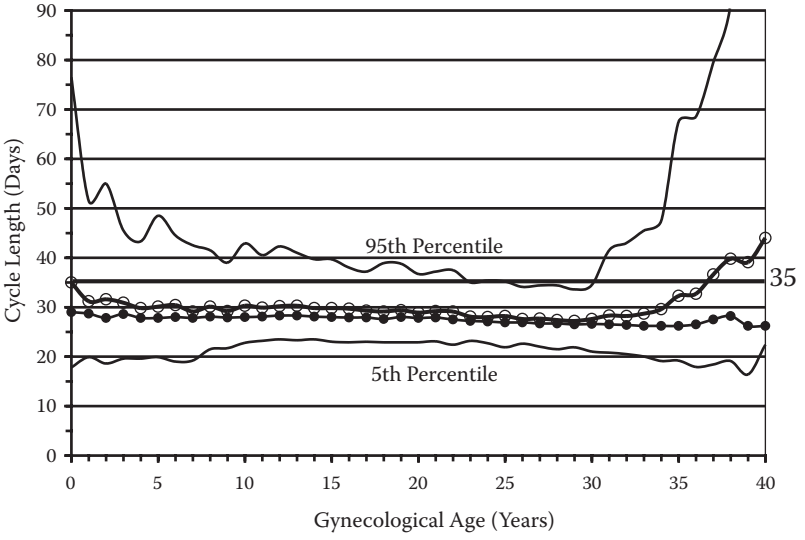

FIGURE 10.3 Fifth, 50th (median), mean and 95th percentiles of the length of the menstrual cycle by gynecological age (years since menarche) in the general population. (See Vollman, R. F., 1977, *The Menstrual Cycle*, Philadelphia: W.B. Saunders.) Plotting menstrual cycle data relative to gynecological age reduces variance caused by menarche occurring at different calendar ages. (●, median; ○, mean; 35 days = conventional but arbitrary defining criterion for oligomenorrhea.)

Women menstruate for about 40 years. The median age at which the first menstrual cycle occurs (i.e., *menarche*) has declined from ~17 years to ~12.8 years over the past 150 years in the United States and other developed countries, and similarly over the past 40 years in China* (Parent et al. 2003). *Primary amenorrhea* (i.e., a delay of menarche caused by some external factor or medical condition) is usually defined statistically as the occurrence of menarche more than 2.5 standard deviations above the average age of menarche in the population. At present, this definition corresponds to 15 years in the United States. Because this definition is statistical, about 1% of girls satisfy the definition even though they are developing at their normal rate without any external factor or medical condition (Chumlea et al. 2003). In large studies of the general population (Pettersson et al. 1973; Singh 1981; Bachmann and Kemmann 1982), *secondary amenorrhea* has been defined as the cessation of menstrual cycles for at least 90 days sometime after menarche. These studies have found the prevalence of secondary amenorrhea in the general population to range from 2 to 5%.

In a "normal" probability distribution, the mean and median (i.e., the 50th percentile) are equal. Because menstrual cycles can be much longer than short cycles can be short, however, the mean length of menstrual cycles in the general population is longer, and at certain ages much longer, than the median length. The median length of menstrual cycles declines slowly and progressively from ~30 to ~27 days over the entire 40-year reproductive lifetime of women (Figure 10.3), but the mean length declines from ~35 days to ~30 days in just the first 5 years after menarche, and then continues to decline slowly for the next 25 years, before rising rapidly to ~45 days during the last decade before the last menstrual cycle (i.e., *menopause*).

Month-to-month variability in the lengths of menstrual cycles also displays a U-shaped variation over the reproductive life span. The 5th to 95th percentile range in cycle lengths declines rapidly from ~60 days to ~20 days during the first decade after menarche (Figure 10.3). The range then declines further but slowly to ~12 days over the next 20 years before increasing rapidly to ~140 days during the last decade before menopause. For this reason, the incidence of oligomenorrhea also varies greatly with age, being much higher in the first and fourth decades of *gynecological age* (i.e., years after menarche).

In addition to perceptible variations in quantity (i.e., length), menstrual cycles vary imperceptibly in quality (i.e., fertility). Menstrual cycles can be either ovulatory (i.e., releasing an egg capable of fertilization) or anovulatory. The follicular phase can also be prolonged with deficient estrogen, while the luteal phase is shortened with deficient progesterone, without any change in the length of the menstrual cycle. Furthermore, even ovulation and fertilization are unproductive if a short or progesterone-deficient luteal phase makes the uterus inhospitable to the implantation of a fertilized egg. Affected women are unaware of these conditions until they undergo an endocrine workup in which hormones are measured in blood, urine, or saliva samples. Anovulation and short luteal phase are very common during adolescence (Figure 10.4).

* A discussion of the reasons hypothesized for this trend is beyond the scope of this chapter. The reader is directed to the paper by Parent et al. (2003) for more information.

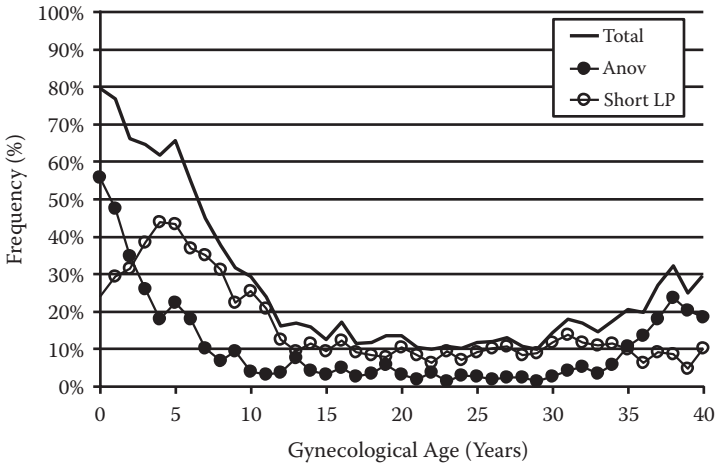

FIGURE 10.4 Variation of the incidence of short luteal phase (SLP) and anovulation (Anov) with gynecological age in the general population. (See Vollman, R. F., 1977, *The Menstrual Cycle*, Philadelphia: W.B. Saunders.)

The transition from the unstable adolescent to the stable adult menstrual cycle is subtle, and for most women occurs gradually over 10 to 12 years. In some women, menstrual stability and luteal adequacy develop quickly, while in others they never do. The subtlety of sexual development and the wide variation in adolescent menstrual cycles make research into menstrual disorders in adolescent athletes especially difficult. To avoid confusing the effects of exercise training with the often prolonged time-course of sexual development, any report of the incidence of menstrual cyclicity in adolescent athletes should be compared to the incidence of the same cyclicity in age-matched nonathletes.

MENSTRUAL DISORDERS IN ATHLETES

Menstrual irregularity becomes a menstrual disorder when it compromises fertility. Research into menstrual disorders in athletes began with survey studies that asked athletes questions about the length and the presence or absence of menstrual cycles. These studies found a high prevalence of *clinical* (i.e., perceptible or symptomatic) menstrual disorders (oligomenorrhea and amenorrhea) and inspired several speculations about the cause of these disorders. However, questionnaires cannot determine the prevalence of menstrual disorders that have no overt or perceptible signs. Therefore, the survey studies were followed by observational studies that measured imperceptible qualities, such as ovulation and hormone concentrations. These observational studies found a high prevalence of *subclinical* menstrual disorders (anovulation and luteal phase deficiency) among eumenorrheic athletes, and also contradicted certain hypotheses inspired by the surveys. However, observational studies are themselves limited in that they collect data after effects have already occurred, which prevents them from definitively determining what preceding event

had caused the effects. To determine causation, data must be collected before and after controlled doses of hypothetical causal factors are administered to experimental subjects. Therefore, the observational studies of athletes were followed by prospective, controlled experiments that could demonstrate causal relationships by inducing, preventing, and reversing effects on reproductive function.

Surveys of Clinical Menstrual Disorders

Most surveys of clinical menstrual disorders among female athletes have found higher prevalences in those participating in aesthetic (e.g., gymnastics, dancing), endurance (e.g., running), and weight-class (e.g., lightweight rowing, martial arts) sports. Prevalence tended to be higher among athletes who were less than 15 years of gynecological age. The disorders were associated with various factors in the athletic lifestyle, such as body size, body composition, exercise training volume, energy intake, and energy expenditure. These findings led some investigators to speculate that one or more of these associated factors might cause the disorders. Meanwhile, others speculated that menstrual disorders in athletes might be like other types of infertility: perhaps participation in sports virilized women by elevating their androgen levels (Baker et al. 1981), or perhaps it increased breast motion and elevated prolactin levels as suckling does in nursing mothers (Brisson et al. 1980), or perhaps exercise was a stress that elevated cortisol and induced infertility as it had done in animal experiments (Selye 1939).

The associated factor most widely popularized to explain the high prevalence of menstrual disorders in athletes was a decline in body fat below a critical level. Interpreted as the amount of stored energy needed to maintain a future pregnancy, the critical level was also postulated to be the amount of adipose tissue needed to convert androgens in the bloodstream to estrogens (Frisch 1994), even though this conversion also takes place in muscle (Longcope et al. 1969), and athletes typically have more muscle than nonathletes. The body fat hypothesis was later contradicted by surveys that did not find the proper temporal relationship between changes in body composition and menstrual function (i.e., changes in menstrual function occurred before changes in body composition), and did not consistently verify the association of menstrual status with body composition (Redman and Loucks 2005). Eumenorrheic and amenorrheic athletes were found to span a common range of body composition leaner than most eumenorrheic sedentary women.

Primary Amenorrhea

In contrast to the 1% prevalence by definition of primary amenorrhea in the general population (Chumlea et al. 2003), surveys found a prevalence of more than 22% in cheerleading, diving, and gymnastics (Beals and Manore 2002). The age of menarche in athletes has been extensively reviewed (Scott and Johnston 1982; Malina 1983; Malina et al. 1994). Some retrospective surveys found the age of menarche in athletes who begin training before menarche to be later than in those who begin training afterward, and these reports may have led to the belief that menarche is delayed by intense exercise during childhood. This belief may be incorrect, though, because an elegant study has shown such retrospective surveys to be inherently

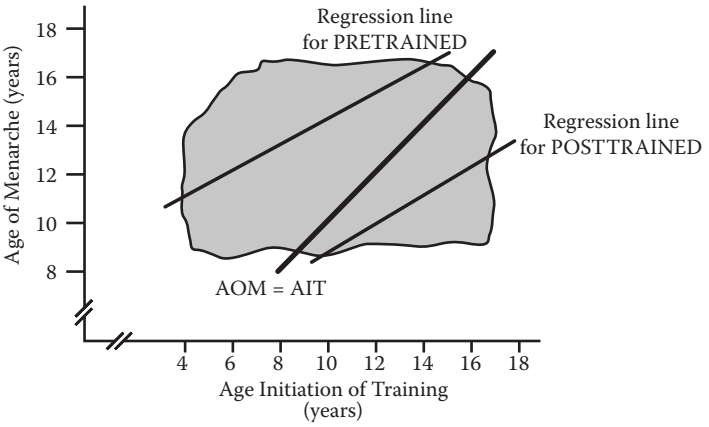

FIGURE 10.5 Scatter plot of the artifactual relationship between age of menarche (AOM) and age of initiation of training (AIT) when random data are divided by the identity line AOM = AIT. (Stager, J. M., Wigglesworth, J. K., and Hatler, L. K., 1990, Interpreting the Relationship between Age of Menarche and Prepubertal Training, *Med Sci Sports Exerc*, 22, 54–58. With permission.)

biased (Stager et al. 1990). In this study, artificial data for 30,000 "athletes" were computer generated with a random relationship between age of menarche (AOM) and age at initiation of training (AIT) (Figure 10.5). The data were then divided by the identity line AOM = AIT into two groups with AIT < AOM and AIT > AOM. Despite the random relationship between the two variables, statistical analysis found AOM to be significantly correlated with AIT ($r = 0.4$, $p < 0.001$) in each group, and AOM to be higher in AIT < AOM than in AIT > AOM (AOM = 13.9 versus 11.7 years, $p = 0.001$). Thus, dividing respondents to retrospective surveys into two groups by means of the identity line AOM = AIT ensures that the group with AIT < AOM will be deficient in individuals with low AOM, while the group with AIT > AOM is deficient in individuals with high AOM. As a result, the results of such studies are not credible.

On the other hand, animal experiments have demonstrated persuasively that puberty can be delayed by various means, including intense exercise, although all of these means may have had their effect by lowering energy availability. Nevertheless, the apparent relationship between childhood athletics and age of menarche can be attributed to selection and socialization without invoking any physiological mechanism (Malina 1983). Due to their more linear physique, girls who mature at an older age tend to perform better at motor tasks than their age-matched postmenarcheal peers, and this may encourage them to continue participating in sports. By contrast, early maturing girls may be diverted away from sports by new social roles and changing interests. Therefore, on the basis of the currently available data, it is correct to state that on average female athletes achieve menarche at an older age than nonathletes, but we lack evidence confirming that this phenomenon is a direct effect of their athletic training. The specific mechanisms responsible for the older age of menarche in female athletes remain unknown.

Secondary Amenorrhea

The prevalence of secondary amenorrhea among female athletes has been found to vary widely with sport, age, training volume, and body weight (Redman and Loucks 2005). In endurance, aesthetic and weight-class sports, it can be as much as 10 times higher than in the general population (Nattiv et al. 2007). It has been reported to be as high as 69% in dancers (Abraham et al. 1982) and 65% in long-distance runners (Dusek 2001). Among runners, prevalence has been found to increase from 3 to 60% as training mileage increased from <13 km (8 miles) to >113 km (70 miles) per week and as body weight decreased from >60 to <50 kg (Sanborn et al. 1982), and also from 9% in those older than 15 years of gynecological age to 67% in those who were younger (Baker et al. 1981).

The prevalence estimates described above should be viewed with caution, because the studies from which they are derived often suffered from methodological limitations. For example, because the prevalence of menstrual disorders is higher in adolescents than in adults, some studies may have overestimated the impact of exercise training on the prevalence of menstrual disorders in adolescent athletes by comparing them to adults instead of to a gynecologically age-matched control group. Others characterized a small number of athletes on a single athletic team who may not have been representative of athletes in general, or even of other teams in the same sport. In addition, because memory is unreliable, studies based on recollections of menstrual cycle dates are less reliable than prospective records for assessing menstrual regularity.

Observational Studies of Menstrual Disorders

By comparing hormone measurements in amenorrheic and eumenorrheic athletes to those in eumenorrheic sedentary women, observational studies were able to test some hypotheses inspired by the results of survey studies. Speculations about exercise disrupting the female reproductive system by inducing androgen, prolactin, and large cortisol responses to exercise were contradicted by findings of smaller responses in amenorrheic athletes than in eumenorrheic athletes (Loucks and Horvath 1984; De Souza et al. 1991, 1994).

Clinical Menstrual Disorders

Athletes with secondary amenorrhea were found to produce low levels of estrogen and progesterone every day, indicating a complete absence of follicular development, ovulation, and luteal function (Figure 10.2) (AA) (Loucks et al. 1989). The proximal cause of this infertility was found to be a disruption of the pulsatile rhythm of LH (Veldhuis et al. 1985; Yahiro et al. 1987; Loucks et al. 1989; Laughlin and Yen 1996). Amenorrheic athletes displayed few LH pulses at irregular intervals (Figure 10.1 (AA). Administration of GnRH demonstrated that the disruption of LH pulsatility was caused by disruption of GnRH pulsatility and not by a pituitary disorder (Veldhuis et al. 1985; Loucks et al. 1989). This differential diagnosis classified the amenorrhea as *functional hypothalamic amenorrhea*.

The mechanisms by which the GnRH pulse generator is disrupted continue to be the focus of much research, including investigations of the hormonal receptors

on GnRH neurons in the arcuate nucleus of the hypothalamus, the signaling pathways to GnRH neurons from other centers in the hypothalamus and elsewhere in the brain, and responses of glucose and lipid-sensing neurons to metabolite concentrations in the blood, as well as effects of pharmacological and physiological treatments on intermediate signals and HPO axis function (Chow et al. 2008; Webster and Tsutsumi 2009; Tena-Sempere et al. 2010).

Subclinical Menstrual Disorders

By measuring sex steroids in blood or sex steroid metabolites in saliva or urine sampled daily throughout a menstrual cycle, observational studies found subclinical menstrual disorders to be common among both highly trained (Loucks et al. 1989) and recreational (Ellison and Lager 1986; De Souza et al. 1998) eumenorrheic athletes in some sports. Luteal suppression was detected in women running recreationally as little as 12 miles per week (Ellison and Lager 1986). In one study, 78% of eumenorrheic runners were luteally suppressed or anovulatory (De Souza et al. 1998). Even runners and triathletes with very regular cycles every 26 to 32 days displayed extended follicular phases and abbreviated luteal phases with blunted progesterone levels (Figure 10.2) (CA) compared to equally regular cyclic sedentary women (Figure 10.2) (CS) (Loucks et al. 1989). As in amenorrheic athletes, the proximal cause of subclinical menstrual disorders in these athletes was a disruption of the pulsatile rhythm of LH. Compared to regularly menstruating sedentary women, athletes with subclinical menstrual disorders displayed a slower, but regular, rhythm of larger LH pulses (Figure 10.1) (CA).

On the basis of such observational studies, menstrual status in female athletes can be said to range from eumenorrhea (regularly menstruating and ovulating) to amenorrhea (not menstruating or ovulating), with apparent intermediate levels of subclinical disturbances that may also compromise fertility. However, such studies cannot reveal whether an individual actually does progress sequentially through these levels of disturbance—that is, whether luteal suppression is a mild dose of amenorrhea or an intermediate condition that would worsen to amenorrhea if these same individuals were to further compromise their energy availability by increasing their exercise training workload or reducing their energy intake. Alternatively, luteal suppression and amenorrhea may both be end points of athletic training in individuals whose reproductive systems are more and less robust, respectively, against the influence of athletic training.

Cross-sectional comparisons of estimated energy availability in runners suggested that their clinical and subclinical menstrual disorders might be caused by low energy availability (Figure 10.6) (Loucks 2007). Surprisingly, both amenorrheic and eumenorrheic athletes report stable body weights on energy intakes similar to those of sedentary women despite much higher energy expenditures. Extensive observational data on the energy and carbohydrate intakes of athletes in many sports indicated that, with the notable exception of cross-country skiers, female athletes consume ~30% less energy and carbohydrates than do male athletes when the intakes are normalized for body weight (Burke et al. 2001).

Amenorrheic athletes displayed low levels of plasma glucose (Laughlin and Yen 1996), insulin (Laughlin and Yen 1996), insulin-like growth factor I (IGF-I) (Zanker

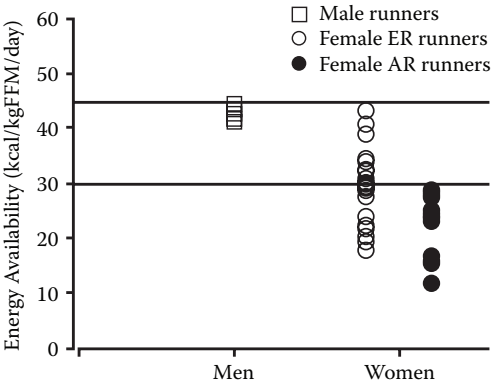

FIGURE 10.6 Energy availabilities of adult long-distance runners. Four studies of male runners, 25 studies of female eumenorrheic (ER) runners, and 12 studies of female amenorrheic (AR) runners provided sufficient information on energy intake, training mileage, and body composition to enable energy availability to be estimated. Running energy expenditure was estimated as 90 kcal/mile. (kgFFM, kilogram of fat-free mass.) (Loucks, A. B., 2007, Low Energy Availability in the Marathon and Other Endurance Sports, *Sports Med*, 37(4–5), 348–352. With permission.)

and Swaine 1998a), the ratio of IGF-I to insulin-like growth factor binding protein-1 (IGF-I/IGFBP-1) (an index of IGF-I bioavailability) (Laughlin and Yen 1996), leptin (Laughlin and Yen 1997; Thong et al. 2000) and triiodothyronine (T_3) (Myerson et al. 1991; Loucks et al. 1992; Zanker and Swaine 1998a,b), as well as low resting metabolic rates (Myerson et al. 1991). They also displayed elevated growth hormone (GH) (Laughlin and Yen 1996) in addition to mildly elevated cortisol levels (Loucks et al. 1989; De Souza et al. 1991; Laughlin and Yen 1996). These are all classic endocrine signs of energy deficiency: low T_3 indicating a compensatory suppression of metabolic rate, low insulin indicating a shift in metabolic fuels from glucose to fatty acids, an elevated ratio of cortisol to insulin indicating accelerated proteolysis for gluconeogenesis, and an elevated ratio of GH to IGF-1 indicating hepatic resistance to protein synthesis. Luteally suppressed eumenorrheic athletes displayed similar but less extreme endocrine abnormalities (Loucks et al. 1989; Laughlin and Yen 1996, 1997; De Souza et al. 2003). Thus, metabolic substrates and hormones told a consistent, dose-responsive story of chronic energy and carbohydrate deficiency in female athletes.

Prospective Experiments

Further Contradictions of the Body Fat Hypothesis

When the growth and sexual development of young rats was blocked experimentally by dietary restriction, normal LH pulsatility was found to resume only a few hours after *ad libitum* feeding was permitted, long before any change in body mass or composition (Bronson 1986). Subsequently, it was also noticed that after surgery reduced the amount of food that severely obese women (body weight ~130 kg; BMI ~47)

could eat, amenorrhea occurred while the women were still obese (body weight ~97 kg; BMI ~35) (DiCarlo et al. 1999). Nevertheless, interest in the body fat hypothesis was rejuvenated in the mid-1990s with the discovery of the hormone leptin and the location of leptin receptors in the hypothalamus.

Synthesized and secreted by adipose tissue, leptin was originally thought to communicate information about fat stores. Later, it was found to vary profoundly in response to fasting, dietary restriction, refeeding after dietary restriction, and overfeeding before any changes in adiposity occurred. This led to the hypothesis that leptin also signals information about dietary intake, and specifically carbohydrate intake after leptin synthesis was found to be regulated by the tiny flux of glucose through the hexosamine biosynthesis pathway in both muscle and adipose tissue. Then, it was found that eumenorrheic and amenorrheic athletes were distinguished not by different 24-hour mean concentrations of leptin, but rather by different amplitudes in the diurnal rhythm of leptin (Laughlin and Yen 1997), and that this diurnal rhythm depends not on energy intake but rather on energy availability, or more specifically on carbohydrate availability (Hilton and Loucks 2000). Thus, if low leptin levels disrupt the GnRH pulse generator in exercising women, they are more likely to do so as a result of low energy or carbohydrate availability than as a signal of low energy stores. The current consensus is that leptin plays an indirect permissive (i.e., necessary but insufficient) role, mediated by kisspeptin neurons (Tena-Sempere et al. 2010), in puberty onset and fertility.

Experimental Evidence for the Exercise Stress Hypothesis

According to the exercise stress hypothesis, exercise constitutes a stress that disrupts the GnRH pulse generator by activating the hypothalamic-pituitary-adrenal (HPA) axis. This hypothesis was supported by considerable animal research demonstrating that GnRH neurons are disturbed by activation of the HPA axis via pathways involving corticotropin-releasing hormone, endogenous opioid and pro-opiomelanocortin-derived peptides, and cortisol negative feedback. Early experiments by Hans Selye in the 1930s (Selye 1939) and by others later (Asahina et al. 1959; Axelson 1987; Chatterton et al. 1990; Manning and Bronson 1989, 1991) induced anestrus and ovarian atrophy in rats by abruptly forcing them to run or swim for prolonged periods. Cortisol levels elevated by hundreds of percent were interpreted as evidence of "stress," and the induced anestrus was interpreted as evidence that exercise stress had a counterregulatory influence on the female reproductive system. Elevations of resting serum cortisol levels in amenorrheic and eumenorrheic athletes and in anorexia nervosa patients (Ding et al. 1988; Loucks et al. 1989; De Souza et al. 1991, 1994) were interpreted by some as confirming this hypothesis, even though the elevations were only mild (10 to 30%).

In fact, only one experiment had successfully employed exercise to induce menstrual disorders in eumenorrheic women (Bullen et al. 1985), and it may have been confounded by energy deficiency. In this experiment, habitually sedentary women were acutely exposed to a high volume of aerobic exercise in imitation of Selye's experiments on rats. A large proportion of menstrual disorders, mostly luteal suppression, occurred in the first month, and an even larger proportion, mostly

anovulation, occurred in the second. These disorders were more prevalent in a subgroup fed a weight-loss diet than in another subgroup fed for weight maintenance. Even the weight maintenance subgroup may have been underfed, however, because behavior modification and endocrine-mediated alterations in resting metabolic rate can counteract the potential influences of dietary energy excess or deficiency on body mass (Leibel et al. 1995). Thus, the possibility remained that the menstrual disorders may have been caused by a failure to increase energy intake to compensate for the increased energy cost of exercise (i.e., low energy availability), rather than by exercise.

The first evidence contrary to the stress hypothesis appeared when glucose administration during exercise was found to prevent the cortisol response to exercise in both rats (Slentz et al. 1990) and men (Tabata et al. 1991). These findings broke a crucial link in the logical chain of deduction between exercise and menstrual disorders by showing that the rise in cortisol levels during prolonged exercise is not caused by exercise but rather by acute energy or carbohydrate deficiency. In the light of these experimental results, all of the previous animal and human investigations of the influence of the "activity stress paradigm" on reproductive function were seen to have confounded the "stress" of exercise with low energy availability (e.g., by requiring animals to run farther and farther for smaller and smaller food rewards). Even Selye's experimental animals, whose ovaries were interpreted to have atrophied due to "stress," had displayed classic symptoms of starvation, including shrinkage of the liver, loss of muscular tone, erosions of the digestive tract, a fall in body temperature, and the disappearance of adipose tissue.* Furthermore, because cortisol is a glucoregulatory hormone secreted in response to low blood glucose levels, the mildly elevated cortisol levels seen in female athletes could also be interpreted as a response to chronic energy deficiency. Thus, by the early 1990s the experimental evidence in favor of the exercise stress hypothesis had been severely weakened, but the definitive experiment to prove it wrong had yet to be performed.

Experimental Evidence for the Energy Availability Hypothesis

Contrary to the exercise stress hypothesis, the energy availability hypothesis postulates that energy expended in one physiological function, such as locomotion, is unavailable for others, such as reproduction. More specifically, the hypothesis holds that failure to provide the body with sufficient metabolic fuels (especially carbohydrates to meet the glucose requirements of the brain) causes an alteration in brain function that disrupts the hypothalamic GnRH pulse generator.

Abundant data from biological field trials support this hypothesis. In rodents, anestrus has been induced by food restriction, the administration of pharmacological blockers of carbohydrate and fat metabolism, insulin administration (which shunts metabolic fuels into storage), and cold exposure (which consumes metabolic fuels via thermogenesis) (Wade and Schneider 1992). Moreover, the disruptive effects on the reproductive system seen under these conditions were independent of body size and composition. Animal research also indicated that GnRH neuron activity and LH

* For a general criticism of stress as a useful concept in biology, see Loucks, A. (2009), Is Stress Measured in Joules? *Military Psychology* 21(S1): S101–S107.

pulsatility are regulated by brain glucose availability via two separate mechanisms involving the area postrema in the caudal brain stem and the vagus nerve (Wade et al. 1996). Together with the observations of metabolic substrates and hormones in female athletes, this animal research suggested that combinations of dietary restriction and exercise energy expenditure might reduce the amount of energy in general, and glucose in particular, available for maintaining normal function of the GnRH pulse generator.

The Definitive Tests of the Exercise Stress and Energy Availability Hypotheses

The controversy over whether the stress of exercise or low energy availability disrupts reproductive function in the Female Athlete Triad was resolved by an experiment that determined the independent effects of exercise stress and energy availability on LH pulsatility (Loucks and Heath 1994; Loucks et al. 1998). Energy availability was defined, measured, and controlled operationally as dietary energy intake minus exercise energy expenditure. In the absence of any widely accepted, empirically operational definition of stress (Loucks 2009), exercise stress was defined as everything associated with exercise, except its energy cost. Habitually sedentary, regularly menstruating women were assigned to sedentary or exercising groups and administered both balanced (45 kcal/kg/FFM) and low energy availability (10 kcal/kg/FFM) treatments. Low energy availability suppressed LH pulse frequency, regardless of whether the low energy availability was caused by dietary energy restriction or exercise energy expenditure. In the exercising women, disruption of LH pulsatility was prevented by increasing their energy intake in compensation for the energy they expended in exercise, demonstrating that exercise has no suppressive effect on hypothalamic regulation of the HPO axis beyond the impact of its energy cost. Low energy availability also suppressed T_3, insulin, IGF-I, and leptin while increasing GH and cortisol (Loucks and Heath 1994; Loucks et al. 1998; Hilton and Loucks 2000) in a pattern reminiscent of amenorrheic and luteally suppressed eumenorrheic athletes.

Later, the energy availability and exercise stress hypotheses were further tested by two animal experiments that examined effects on HPO axis target organs. Amenorrhea was induced in monkeys by training them to run voluntarily on a motorized treadmill for longer and longer periods while their food intake remained constant (Williams, Caston-Balderrama et al. 2001). The monkeys became amenorrheic abruptly in 7 to 24 months after one or two cycles of luteal suppression. When the diet of half of the monkeys was then supplemented without any moderation of their exercise regimen, their menstrual cycles were restored with a speed that was directly related to the number of calories consumed (Williams, Helmreich et al. 2001). Subsequently, a novel animal model of the entire Female Athlete Triad was developed (DiMarco et al. 2007). In that model, rats were habituated to voluntary wheel running for 90 days and then randomized to control and 30% restricted diets for the next 90 days. Even though both groups ran similar distances and expended similar amounts of energy in running, estradiol was suppressed, estrous cycling ceased, ovaries were atrophied, and the bone mineral content of the femur and tibia were reduced only in the underfed rats.

Discovery of the Energy Availability Threshold

The dose-response dependence of LH pulsatility on energy availability in exercising women has also been determined (Loucks and Thuma 2003). LH pulse frequency was

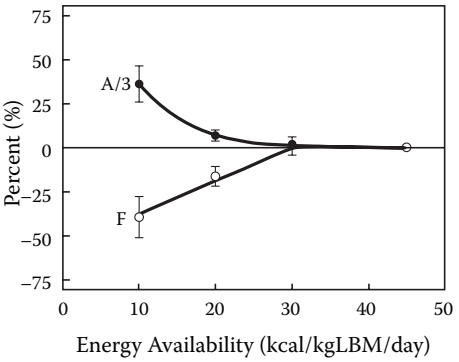

FIGURE 10.7 Incremental effects of energy availability on LH pulse amplitude (A/3) and LH pulse frequency (F). Effects are expressed relative to values at 45 kcal/kgLBM/day. Effects on LH pulse amplitude have been divided by three for graphical symmetry. As energy availability declines from energy balance at approximately 45 kcal/kgLBM/day, effects begin at a threshold at approximately 30 kcal/kgLBM/day and become more extreme as energy availability is further reduced below 20 kcal/kgLBM/day. (Loucks, A. B., and Thuma, J. R., 2003, Luteinizing Hormone Pulsatility Is Disrupted at a Threshold of Energy Availability in Regularly Menstruating Women, *J Clin Endocrinol Metab*, 88(1), 297–311. With permission.)

suppressed and pulse amplitude was increased below a threshold of energy availability at ~30 kcal/kgFFM/day (Figure 10.7). The maintenance of normal LH pulsatility in this short-term experiment despite a restriction of energy availability from 45 to 30 kcal/kgFFM/day suggests that the regulation of the reproductive system in women might be robust against reductions in energy availability as large as 33%. In particular, athletes may be able to prevent menstrual disorders by maintaining energy availabilities above that threshold. Because the total energy expenditure during exercise in this experiment was ~840 kilocalories, these results suggest that women may be able to maintain normal LH pulsatility while running up to 8 miles a day as long as they do not simultaneously reduce their dietary energy intake below 45 kcal/kgFFM/day, at which habitually sedentary women are in energy balance. If they do reduce their dietary energy intake, as many exercising women do, then they risk falling below the threshold of energy availability needed to maintain normal LH pulsatility.

Figure 10.8 shows the associated dose-response effects of energy availability on several metabolic hormones and substrates. Down to 30 kcal/kgFFM/day, the glucoregulatory hormone responses maintained plasma glucose concentrations to within a few percent of energy-balanced levels by mobilizing stored metabolic fuels. Below that threshold, glucose concentrations fell increasingly, despite larger hormone responses and steep increases in fat oxidation and gluconeogenesis, as indicated by the rise in β-hydroxybutyrate. Hepatic secretion of IGF-I became resistant to GH as T_3 levels declined, most abruptly between 20 and 30 kcal/kgFFM/day. Thus, as energy availability declines below 30 kcal/kgFFM/day, glucoregulatory responses are more extreme but less effective at maintaining plasma glucose levels, and the brain becomes progressively more reliant on ketones as an alternative metabolic fuel for energy production.

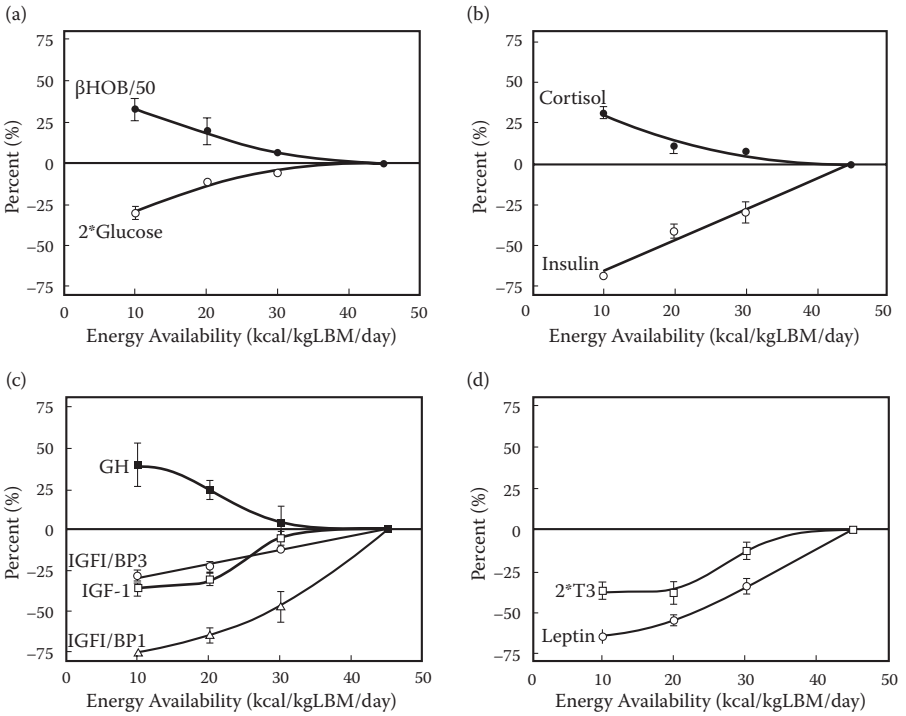

FIGURE 10.8 Incremental effects of restricted energy availability on metabolic substrates and hormones. Effects are shown relative to values at 45 kcal/kgLBM/day. (A) Incremental effects on the metabolic substrates β-HOB (*top*) and plasma glucose (*bottom*). Effects on β-hydroxybutyrate (βHOB) have been divided by 50, and effects on plasma glucose have been doubled for graphical symmetry. (B) Incremental effects on the metabolic hormones cortisol (*top*) and insulin (*bottom*). (C) Incremental effects on the somatotrophic metabolic hormones GH (*top*) and IGF-I (□, *bottom*) and the bioactive ratios IGF-I/IGFBP-1 (△) and IGF-I/IGFBP-3 (○). Both estimates of bioactivity declined significantly and substantially at 30 kcal/kgLBM/day. (D) Incremental effects on the metabolic hormones T_3 (□) and leptin (○). The effect on T_3 is doubled for graphical clarity. (Loucks, A. B., and Thuma, J. R., 2003, Luteinizing Hormone Pulsatility Is Disrupted at a Threshold of Energy Availability in Regularly Menstruating Women, *J Clin Endocrinol Metab*, 88(1), 297–311. With permission.)

Readers are cautioned to take note of the fact that the above experiment was performed on older adolescent (i.e., college-aged), habitually sedentary women who had completed their growth, although not all of their reproductive and skeletal development. Similar experiments have not been performed on younger, growing adolescents to determine what their thresholds of energy availability may be.

The Insensitivity of Reproductively Mature Women to Low Energy Availability

The declining incidence of menstrual disorders during adolescence is accompanied by an increase in fertility rates (Ellison 1994). No mechanism of this gradual and

prolonged "maturation" of the HPO axis had ever been proposed, but one was suggested by the finding that calcium balance, which is an index of growth, does not decline to zero until 14 years of gynecological age (Weaver et al. 1995), in parallel with the gradual decline in menstrual disorders. A recent experiment investigated whether the declining incidences of menstrual disorders during adolescence might be mediated by a declining sensitivity of LH pulsatility to low energy availability as the energy cost of growth decreases (Loucks 2006). Contrasting balanced and low energy availabilities (45 and 10 kcal/kgFFM/day) were administered to healthy, habitually sedentary, regularly menstruating, adolescent women (5 to 8 years of gynecological age, ~20 years of calendar age) and adult women (14 to 18 years of gynecological age, ~29 years of calendar age) for 5 days. Low energy availability suppressed LH pulsatility in the adolescents but not in the adults, even though metabolic and endocrine signals of energy deficiency (i.e., plasma glucose, β-hydroxybutyrate, insulin, cortisol, T_3, leptin, IGF-1, and GH) were altered as much or more in the adults as in the adolescents (Loucks 2006).

This insensitivity of LH pulsatility to energy deficiency in reproductively mature women has since proven to be predictive of a corresponding insensitivity of ovarian function to low energy availability (Williams et al. 2010). In a prospective experiment, the energy availability of women 25 to 40 years of age was reduced to ~25 kcal/kgFFM/day by a combination of dietary restriction (~600 kcal/day) and exercise (~200 kcal/day). After 4 months of this treatment, body fatness declined from 32 to 27%, confirming the low energy availability of the women, but they experienced no more than a mild suppression of luteal function.

The relative insensitivity of the adult reproductive system to low energy availability may result from a greater availability of glucose to the brain in adults than in adolescents at identical energy availabilities, because peripheral tissues in full-grown adults do not compete as strongly against the brain for available energy. Alternatively, the gradual desensitization of the reproductive system to energy deficiency during adolescence may be caused by a progressive decline in the sensitivity of central nervous system sensors to signals of energy deficiency. Yet another possibility is that GH, which responded even more strongly to low energy availability in adults than in adolescents (Loucks 2006), may mobilize more free fatty acids from adipose tissue in adults than it does in adolescents. In addition to serving as an energy source for peripheral tissues, long-chain fatty acids cross the blood-brain barrier in the hypothalamus, where they function as an anorexic signal opening potassium channels and reducing the firing rate of neurons that stimulate hepatic glucose production and feeding (Obici et al. 2002). Such neurons may also influence GnRH pulsatility. All of these hypotheses remain to be investigated.

To sum up 30 years of research, the menstrual disorders in the Female Athlete Triad are functional hypothalamic menstrual disorders. These disorders manifest themselves clinically as oligomenorrhea and amenorrhea or subclinically as anovulation and luteal phase deficiency. It appears as though the causative factor is low energy availability induced by increasing exercise energy expenditure or decreasing energy intake. In habitually sedentary, adult women (<15 years of gynecological age), reproductive function is disrupted when energy availability declines below 30 kcal/kgFFM/day, while more mature women appear to be less sensitive to energy deficiency.

TREATMENT OF MENSTRUAL DISORDERS

The treatment of the menstrual disorders in the Female Athlete Triad should proceed in three steps: differential diagnosis of the menstrual disorder, identification of the origin of low energy availability, and behavior modification to increase energy availability.

Because any of the many causes of menstrual disorders can be present in an athlete, the first step in the treatment of any athlete with a menstrual disorder is a differential diagnosis by a qualified physician to exclude pregnancy, lactation, pituitary tumors, mental illnesses, polycystic ovary disease, and a long list of other organic diseases unrelated to low energy availability which also display menstrual symptoms. Through self-selection, menstrual disorders caused by factors unrelated to low energy availability may be present among athletes in disproportionately large numbers, if these factors favor athletic success. For example, the amenorrhea and oligomenorrhea in polycystic ovary disease are frequently accompanied by an excess secretion of androgens. Therefore, an increased incidence of polycystic ovary disease is to be expected in power sports in which high levels of androgens favor success. Polycystic ovary disease may be underdiagnosed among athletes or misdiagnosed as the Triad. About one-third of infertility patients at a major gynecological clinic who had been diagnosed with functional hypothalamic amenorrhea were later found to have underlying polycystic ovary disease that emerged when the patients' diets were improved (Sum and Warren 2009). The cause of infertility in these women oscillated back and forth between functional hypothalamic amenorrhea and polycystic ovary disease as they went on and off of energy-restrictive diets.

For an athlete diagnosed with a functional hypothalamic menstrual disorder, the first step in treatment is to assess her energy availability to determine if it is low. This assessment can be accomplished by having the athlete complete a dietary intake and exercise log. If low energy availability is present, the next step is to identify the origin of her low energy availability with the aim of correcting it. Chapter 9 provides detailed information on both the causes of and interventions for low energy availability, so it will not be reiterated here. Suffice it to say that nutritional counseling in clinical trials has restored spontaneous menstrual cycles in 75% of women with functional hypothalamic amenorrhea within 5 months (Berga et al. 2003).

Although frequently prescribed, neither hormone replacement therapy (HRT) nor oral contraceptives (OCs) have proven effective for restoring spontaneous menstrual cycles in women with functional hypothalamic amenorrhea, because they do not correct the metabolic abnormalities caused by low energy availability. After 8 years of such treatment (Falsetti et al. 2002), 30% of 93 women with functional hypothalamic amenorrhea without an eating disorder remained amenorrheic. HRT had no benefit and OCs delayed and reduced the likelihood of recovering menses. All of those whose body mass index (BMI) increased recovered, whereas none of those whose BMI decreased recovered.

Leptin has also been proposed as a future treatment for hypothalamic amenorrhea, despite leptin's merely permissive role in the regulation of GnRH pulsatility. A 3-month open-label clinical trial of leptin found that ovulation occurred in three of eight (37.5%) women with hypothalamic amenorrhea (Welt et al. 2004). The

treatment degraded nutrition, however, as the anorexigenic (i.e., appetite-suppressing) effect of leptin caused the eight women to lose an average of 4.5% of their body weight and 19% of their body fat during the trial. Nevertheless, these results led to a subsequent 9-month double-blind randomized trial on a second group of women with hypothalamic amenorrhea. The dosage of leptin administered in this trial raised leptin levels in the blood more than 10-fold from (mean ± SD) 4.6 ± 2.0 ng/ml in the lower portion of the normal range (7.4 ± 3.7 ng/ml) for women with similar BMI (18 to 25 kg/m^2) to 59 ± 37 ng/ml (Chou et al. 2011). Menstrual cycles occurred intermittently, with the number menstruating fluctuating from month to month between 3 of 10 (30%) and 4 of 7 (57%). Meanwhile, dosage had to be reduced or withdrawn from 4 of 10 subjects due to excessive weight loss. Therefore, the efficacy and safety of leptin administration as a treatment for hypothalamic amenorrhea is, at best, unclear.

SUMMARY

The length and variability of menstrual cycles are not normally distributed around mean values over the complex 40-year reproductive life span of women, or even during any one of those years, and especially not during the first and fourth of those decades. In addition to varying in quantity (i.e., length), menstrual cycles also vary in quality (i.e., fertility). Even 28-day menstrual cycles can be infertile if ovulation does not occur or the luteal phase is deficient. Menstrual irregularity becomes a menstrual disorder when it compromises fertility. Functional hypothalamic amenorrhea compromises skeletal health and development as well as fertility.

Among female athletes participating in endurance, aesthetic, and weight-class sports, clinical (i.e., symptomatic) and subclinical (i.e., asymptomatic) menstrual disorders are common. Of these, the functional hypothalamic menstrual disorders are believed to be caused by low energy availability due to a large reduction in dietary energy intake or a large increase in exercise energy expenditure. Pharmacological restoration of menstrual cycles with oral contraceptives does not correct the metabolic disorders that accompany such disorders. Conversely, dietary reform will not correct menstrual disorders that are unrelated to low energy availability. Therefore, the first step in treating an athlete with a menstrual disorder is a differential diagnosis of the disorder by a physician to determine the cause so that the appropriate treatment can be prescribed.

For athletes diagnosed with functional hypothalamic menstrual disorders, the second step in treatment is to assess energy availability and, if low energy availability is present, to determine its cause. Once the cause of the low energy availability is determined, then appropriate treatment to increase energy availability can be implemented. In all cases, the goal of treatment is to restore reproductive function by increasing energy availability to a level that restores menstrual function.

REFERENCES

Abraham, S. F., P. J. Beumont, et al. (1982). Body weight, exercise and menstrual status among ballet dancers in training. *Br J Obstet Gynaecol* 89(7): 507–510.

Asahina, K., F. Kitahara, et al. (1959). Influence of excessive exercise on the structure and function of rat organs. *Jpn J Physiol* 9: 322–326.

Axelson, J. F. (1987). Forced swimming alters vaginal estrous cycles, body composition, and steroid levels without disrupting lordosis behavior or fertility in rats. *Physiol Behav* 41(5): 471–479.

Bachmann, G. A., and E. Kemmann (1982). Prevalence of oligomenorrhea and amenorrhea in a college population. *Am J Obstet Gynecol* 144(1): 98–102.

Baker, E. R., R. S. Mathur, et al. (1981). Female runners and secondary amenorrhea: Correlation with age, parity, mileage, and plasma hormonal and sex-hormone-binding globulin concentrations. *Fertil Steril* 36(2): 183–187.

Beals, K. A., and M. M. Manore (2002). Disorders of the female athlete triad among collegiate athletes. *Int J Sport Nutr Exerc Metab* 12(3): 281–293.

Berga, S. L., M. D. Marcus, et al. (2003). Recovery of ovarian activity in women with functional hypothalamic amenorrhea who were treated with cognitive behavior therapy. *Fertil Steril* 80(4): 976–981.

Brisson, G. R., M. A. Volle, et al. (1980). Exercise-induced dissociation of the blood prolactin response in young women according to their sports habits. *Horm Metab Res* 12(5): 201–205.

Bronson, F. H. (1986). Food-restricted, prepubertal, female rats: Rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone. *Endocrinology* 118(6): 2483–2487.

Bullen, B. A., G. S. Skrinar, et al. (1985). Induction of menstrual disorders by strenuous exercise in untrained women. *N Engl J Med* 312(21): 1349–1353.

Burke, L. M., G. R. Cox, et al. (2001). Guidelines for daily carbohydrate intake: Do athletes achieve them? *Sports Med* 31(4): 267–299.

Chatterton, R. T., Jr., A. L. Hartman, et al. (1990). Exercise-induced ovarian dysfunction in the rat. *Proc Soc Exp Biol Med* 193(3): 220–224.

Chou, S. H., J. P. Chamberland, et al. (2011). Leptin is an effective treatment for hypothalamic amenorrhea. *Proc Natl Acad Sci USA* 108(16): 6585–6590.

Chow, B. K. C., V. H. Y. Lee, et al. (2008). Gonadotropin-releasing hormone: Regulation of the GnRH gene. *FEBS J* 275(22): 5458–5478.

Chumlea, W. C., C. M. Schubert, et al. (2003). Age at menarche and racial comparisons in U.S. girls. *Pediatrics* 111(1): 110–113.

De Souza, M. J., A. A. Luciano, et al. (1994). Clinical tests explain blunted cortisol responsiveness but not mild hypercortisolism in amenorrheic runners. *J Appl Physiol* 76(3): 1302–1309.

De Souza, M. J., M. S. Maguire, et al. (1991). Adrenal activation and the prolactin response to exercise in eumenorrheic and amenorrheic runners. *J Appl Physiol* 70(6): 2378–2387.

De Souza, M. J., B. E. Miller, et al. (1998). High frequency of luteal phase deficiency and anovulation in recreational women runners: Blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. *J Clin Endocrinol Metab* 83(12): 4220–4232.

De Souza, M. J., J. Van Heest, et al. (2003). Luteal phase deficiency in recreational runners: Evidence for a hypometabolic state. *J Clin Endocrinol Metab* 88(1): 337–346.

Di Carlo, C., and S. Palomba, et al. (1999). Hypogonadotropic hypogonadotropism in obese women after biliopancreatic diversion. *Fertil Steril* 72(5): 905–909.

DiMarco, N. M., L. Dart, et al. (2007). Modified activity-stress paradigm in an animal model of the Female Athlete Triad. *J Appl Physiol* 103(5): 1469–1478.

Ding, J. -H., C. B. Scheckter, et al. (1988). High serum cortisol levels in exercise-associated amenorrhea. *Ann Intern Med* 108: 530–534.

Dusek, T. (2001). Influence of high intensity training on menstrual cycle disorders in athletes. *Croat Med J* 42(1): 79–82.

Ellison, P. T. (1994). Advances in human reproductive ecology. *Annu Rev Anthropol* 23: 255–275.

Ellison, P. T., and C. Lager (1986). Moderate recreational running is associated with lowered salivary progesterone profiles in women. *Am J Obstet Gynecol* 154: 1000–1003.

Falsetti, L., A. Gambera, et al. (2002). Long-term follow-up of functional hypothalamic amenorrhea and prognostic factors. *J Clin Endocrinol Metab* 87(2): 500–505.

Frisch, R. E. (1994). The right weight: Body fat, menarche and fertility. *Proc Nutr Soc* 53(1): 113–129.

Hilton, L. K., and A. B. Loucks (2000). Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. *Am J Physiol Endocrinol Metab* 278(1): E43–E49.

Laughlin, G. A., and S. S. C. Yen (1996). Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. *J Clin Endocrinol Metab* 81(12): 4301–4309.

Laughlin, G. A., and S. S. C. Yen (1997). Hypoleptinemia in women athletes: Absence of a diurnal rhythm with amenorrhea. *J Clin Endocrinol Metab* 82(1): 318–321.

Leibel, R. L., M. Rosenbaum, et al. (1995). Changes in energy expenditure resulting from altered body weight. *N Engl J Med* 332(10): 621–628.

Longcope, C., T. Kato, et al. (1969). Conversion of blood androgens to estrogens in normal adult men and women. *J Clin Invest* 48(12): 2191–2201.

Loucks, A. B. (2006). The response of luteinizing hormone pulsatility to five days of low energy availability disappears by 14 years of gynecological age. *J Clin Endocrinol Metab* 91: 3158–3164.

Loucks, A. B. (2007). Low energy availability in the marathon and other endurance sports. *Sports Med* 37(4–5): 348–352.

Loucks, A. (2009). Is stress measured in joules? *Military Psychol* 21(S1): S101–S107.

Loucks, A. B., and E. M. Heath (1994). Dietary restriction reduces luteinizing hormone (LH) pulse frequency during waking hours and increases LH pulse amplitude during sleep in young menstruating women. *J Clin Endocrinol Metab* 78(4): 910–915.

Loucks, A. B., and S. M. Horvath (1984). Exercise-induced stress responses of amenorrheic and eumenorrheic runners. *J Clin Endocrinol Metab* 59(6): 1109–1120.

Loucks, A. B., G. A. Laughlin, et al. (1992). Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. *J Clin Endocrinol Metab* 75(2): 514–518.

Loucks, A. B., J. F. Mortola, et al. (1989). Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. *J Clin Endocrinol Metab* 68(2): 402–411.

Loucks, A. B., and J. R. Thuma (2003). Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab* 88(1): 297–311.

Loucks, A. B., M. Verdun, et al. (1998). Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. *J Appl Physiol* 84(1): 37–46.

Malina, R. M. (1983). Menarche in athletes: A synthesis and hypothesis. *Ann Hum Biol* 10(1): 1–24.

Malina, R. M., R. C. Ryan, et al. (1994). Age at menarche in athletes and their mothers and sisters. *Ann Hum Biol* 21(5): 417–422.

Manning, J. M., and F. H. Bronson (1989). Effects of prolonged exercise on puberty and luteinizing hormone secretion in female rats. *Am J Physiol* 257(6 Pt 2): R1359–R1364.

Manning, J. M., and F. H. Bronson (1991). Suppression of puberty in rats by exercise: Effects on hormone levels and reversal with GnRH infusion. *Am J Physiol* 260(4 Pt 2): R717–R723.

Myerson, M., B. Gutin, et al. (1991). Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. *Med Sci Sports Exerc* 23: 15–22.

Nattiv, A., A. B. Loucks, et al. (2007). American College of Sports Medicine Position Stand: The female athlete triad. *Med Sci Sports Exerc* 39(10): 1867–1882.

Obici, S., Z. Feng, et al. (2002). Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* 51(2): 271–275.

Parent, A. S., G. Teilmann, et al. (2003). The timing of normal puberty and the age limits of sexual precocity: Variations around the world, secular trends, and changes after migration. *Endocr Rev* 24(5): 668–693.

Pettersson, F., H. Fries, et al. (1973). Epidemiology of secondary amenorrhea. I. Incidence and prevalence rates. *Am J Obstet Gynecol* 117(1): 80–86.

Redman, L. M., and A. B. Loucks (2005). Menstrual disorders in athletes. *Sports Med* 35(9): 747–755.

Sanborn, C. F., B. J. Martin, et al. (1982). Is athletic amenorrhea specific to runners? *Am J Obstet Gynecol* 143(8): 859–861.

Scott, E. C., and F. E. Johnston (1982). Critical fat, menarche, and the maintenance of menstrual cycles: A critical review. *J Adolesc Health Care* 2(4): 249–260.

Selye, H. (1939). The effect of adaptation to various damaging agents on the female sex organs in the rat. *Endocrinology* 25: 615–624.

Singh, K. B. (1981). Menstrual disorders in college students. *Am J Obstet Gynecol* 140(3): 299–302.

Slentz, C. A., J. M. Davis, et al. (1990). Glucose feedings and exercise in rats: Glycogen use, hormone responses, and performance. *J Appl Physiol* 69: 989–994.

Speroff, L., and M. A. Fritz (2005). *Clinical gynecologic endocrinology and infertility*. Philadelphia: Lippincott Williams & Wilkins.

Stager, J. M., J. K. Wigglesworth, et al. (1990). Interpreting the relationship between age of menarche and prepubertal training. *Med Sci Sports Exerc* 22(1): 54–58.

Stubbs, R. J., D. A. Hughes, et al. (2004). Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. *Am J Physiol: Regul, Integr Comp Physiol* 286(2): R350–R358.

Sum, M., and M. P. Warren (2009). Hypothalamic amenorrhea in young women with underlying polycystic ovary syndrome. *Fertil Steril* 92(6): 2106–2108.

Tabata, I., F. Ogita, et al. (1991). Effect of low blood glucose on plasm CRF, ACTH, and cortisol during prolonged physical exercise. *J Appl Physiol* 71: 1807–1812.

Tena-Sempere, M., J. M. Castellano, et al. (2010). Kisspeptins: Bridging energy homeostasis and reproduction. *Brain Res* 1364: 129–138.

Thong, F. S., C. McLean, et al. (2000). Plasma leptin in female athletes: Relationship with body fat, reproductive, nutritional, and endocrine factors. *J Appl Physiol* 88(6): 2037–2044.

Treloar, A. E., R. E. Boynton, et al. (1967). Variation of the human menstrual cycle through reproductive life. *Int J Fertil* 12(1 Pt 2): 77–126.

Veldhuis, J. D., W. S. Evans, et al. (1985). Altered neuroendocrine regulation of gonadotropin secretion in women distance runners. *J Clin Endocrinol Metab* 61(3): 557–563.

Vollman, R. F. (1977). *The Menstrual Cycle*. Philadelphia: W.B. Saunders.

Wade, G. N., and J. E. Schneider (1992). Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev* 16(2): 235–272.

Wade, G. N., J. E. Schneider, et al. (1996). Control of fertility by metabolic cues. *Am J Physiol* 270(1 Pt 1): E1–E19.

Weaver, C. M., B. R. Martin, et al. (1995). Differences in calcium metabolism between adolescent and adult females. *Am J Clin Nutr* 61(3): 577–581.

Webster, N. J. G., and R. Tsutsumi (2009). GnRH pulsatility, the pituitary response and reproductive dysfunction. *Endocr J* 56(6): 729–737.

Welt, C. K., J. L. Chan, et al. (2004). Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 351(10): 987–997.

Williams, N. I., A. L. Caston-Balderrama, et al. (2001). Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: Abrupt transition to exercise-induced amenorrhea. *Endocrinology* 142(6): 2381–2389.

Williams, N. I., D. L. Helmreich, et al. (2001). Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. *J Clin Endocrinol Metab* 86(11): 5184–5193.

Williams, N. I., J. L. Reed, et al. (2010). Estrogen and progesterone exposure is reduced in response to energy deficiency in women aged 25–40 years. *Hum Reprod* 25(9): 2328–2339.

Yahiro, J., A. R. Glass, et al. (1987). Exaggerated gonadotropin response to luteinizing hormone-releasing hormone in amenorrheic runners. *Am J Obstet Gynecol* 156(3): 586–591.

Zanker, C. L., and I. L. Swaine (1998a). Bone turnover in amenorrhoeic and eumenorrhoeic women distance runners. *Scand J Med Sci Sports* 8(1): 20–26.

Zanker, C. L., and I. L. Swaine (1998b). Relation between bone turnover, oestradiol, and energy balance in women distance runners. *Br J Sports Med* 32(2): 167–171.

11 Recommendations for Optimizing Bone Strength and Reducing Fracture Risk in Female Athletes

Michelle Barrack

CONTENTS

INTRODUCTION

Bones are structures that make up the skeletal system and serve important mechanical and protective functions in the body. They provide the body with rigidity and shape, protect vital organs, and allow for bimodal locomotion. Bones that contain more mineral per unit area, and are therefore more "dense," and bones with optimal microarchitectural integrity exhibit higher strength and resistance to fracture. Therefore, female athletes with higher bone mineral density (BMD) and optimal bone microstructure will be less likely to sustain a bone injury. Unfortunately, some female athletes exhibit low BMD and increased risk of bone injuries (including stress reactions and fractures) as well as premature osteoporosis. Consuming inadequate

bone-building nutrients (such as calcium and vitamin D) and participating in low-impact, unidirectional (versus high-impact, multidirectional) sports increase the risk of developing low BMD. However, as indicated in Chapter 9, low energy availability, a condition resulting from an intake of energy (calories) that is not enough to support athletes' exercise energy expenditure, serves as the main precursor to the development of low bone mass in the Female Athlete Triad. To optimize bone mass and strength, it is important for female athletes to consume a diet sufficient in calories and essential vitamins and minerals, and participate in bone-building exercises, such as resistance training, jumping exercises, and explosive multiplanar movements. This is particularly important during adolescence and young adulthood, as this is when peak bone mass is established.

This chapter will examine a spectrum of topics associated with bone health in sport. First, the general characteristics of bone structure, metabolism, and development are covered, in addition to methods of assessing bone mass and strength in female athletes. Next discussed are key genetic and lifestyle characteristics that affect the accumulation, maintenance, and microstructural integrity of bone. Last, sport types and factors associated with low bone mass and fracture risk are reviewed, in addition to behavioral recommendations female athletes can employ to improve bone health and continue active and healthy participation in their sport.

CHARACTERISTICS OF BONE

Bones are hard, porous structures composed of an extracellular mineralized matrix, collagen, cells, vessels, and inorganic minerals (Hadjidakis and Androulakis 2006). Ninety-nine percent of the body's calcium, 80 to 90% of its phosphates, 70% of magnesium, and 40 to 50% of its sodium are located in bone tissue (Stipanuk 2000). Minerals in bone are primarily found in a calcium phosphorus crystalline compound called hydroxyapatite (Stipanuk 2000; Hadjidakis and Androulakis 2006; Datta et al. 2008). In the body, bones serve several important mechanical and protective functions; they provide rigidity and shape, protect the organs and other body structures, and allow for bimodal locomotion (Datta et al. 2008).

Bone tissue exists in two forms, a dense, compact type called *cortical* bone, and a soft, spongy type called *trabecular* bone. Cortical bone makes up the outer layer of long bones (such as those of the limbs), and trabecular, also called *cancellous*, bone makes up the soft, spongy inner layer of bones (Borer 2005; Datta et al. 2008). Sites high in trabecular bone include the vertebrae, femoral neck, and wrist. This (trabecular) type of bone exhibits a higher rate of bone turnover than cortical bone and is, therefore, seen as being more metabolically active. Osteogenic, bone-building, cells position themselves along the surface of trabecular bone (Borer 2005; Datta et al. 2008). Interestingly, trabeculae at the end of long bones orient themselves along the trajectory of the highest stress and then thicken to support against a given load (Borer 2005; Datta et al. 2008). This method of adaptation by trabecular bone allows this bone type to modify in strength and density when exposed to an external load or strain, such as from exercise. This explains, in part, why high-impact exercise promotes bone strength gains in female athletes.

Bone strength and resistance to fracture are affected by both *quantitative* and *qualitative* aspects of bone. Bone quantity represents the amount of bone mineral per unit area (expressed in grams per cm^2) or per unit volume (expressed in grams per cm^3). Bone quality refers to the microstructure and geometry of bone. Microstructural assessments measure the arrangement of the trabecular and cortical bone (independent of bone quantity). Geometry refers to the diameter of bone or distribution of bone mass around the central axis (Langton and Njeh 2003). The methods used to measure areal and volumetric bone density and bone quality are described later in the chapter.

STAGES OF BONE DEVELOPMENT

Bone is not static; rather, it is a highly metabolically active tissue that is constantly undergoing demineralization (resorption) and remineralization (formation). This process is referred to as *bone turnover* or *remodeling*. During the years surrounding adolescence, the rate of bone formation outweighs the rate of bone resorption (Heaney et al. 2000). This leads to a significant accrual of bone, which is particular to this stage of life (Heaney et al. 2000).

Longitudinal and cross-sectional studies indicate that young adults reach a "peak" bone mass during the third decade of life, typically between ages 25 and 30 years (Theintz et al. 1992; Ilich et al. 1994; Fonseca et al. 2001; Arabi et al. 2004). Under normal conditions, approximately 40 to 50% of a female's adult bone mineral content is accrued between ages 11 and 18 years, approximately 90% is accumulated by age 16.9 ± 1.3 years, and 99% is gained by age 26.2 ± 3.7 years (Weaver 2002). Nearly half of the bone mineral content accrual occurs during the 1 to 2 years surrounding the time that a girl begins menstruating (Bailey et al. 2000; MacKelvie et al. 2002; Loud and Gordon 2006). After age 16 years, gains in bone mineral content begin to taper, particularly at the lumbar spine (Theintz et al. 1992; Theintz 1994; Sabatier et al. 1999). Due to the timing of these events, the early to mid-adolescent years are often referred to as the "critical window of opportunity" for gaining bone mass. Young girls who either intentionally, due to disordered eating, or inadvertently, due to a lack of awareness of their energy requirements, consume a calorie intake level that is not enough to support their physical exercise activity may disrupt the normal processes of bone mineral accrual. This is due to hormone adaptations that occur as a result of low energy availability and promote anti-osteogenic changes to bone. If prolonged, this can lead to the development of a low peak BMD and lifetime low bone mass.

During the third and fourth decades of life, bone formation approximately equals bone resorption; thus, BMD generally plateaus during this time. Around age 45 to 55 years, women enter menopause, a life stage characterized hormonally by an early rise in plasma follicle-stimulating hormone (FSH), elevated luteinizing hormone (Marques de Aquino et al. 2008), a reduction in estradiol, and a gradual loss of menses (Porterfield 2001; Borer et al. 2005). Prior studies recognize that bone loss corresponds to the onset of menopause, with initial yearly rates of postmenopausal loss ranging from 0.5 to 2.0% (Ahlborg et al. 2003; Borer et al. 2005). Three to

five years after the onset of menopause, the rate of bone loss declines to a level of approximately 0.5% per year. Overall lifetime postmenopausal losses range from 16 to 31% and vary depending on the bone site (Rey et al. 1994). Due to this loss of bone mass that occurs as a normal part of aging, it is recommended that young female athletes maximize their bone mineral gains early in life. This will lower a woman's risk of developing osteoporosis during the years surrounding menopause. Avoiding bone-related injury, osteoporosis, and osteoporosis-related fragility fractures will encourage a lifetime of activity and health among female athletes.

ASSESSMENT OF BONE STRENGTH AND FRACTURE RISK IN FEMALE ATHLETES

In athletes, bone strength and resistance to fracture are evaluated by assessing bone quantity with technology called dual-energy x-ray absorptiometry (DXA). The results of this x-ray scan are used to identify athletes with low bone mass (Lane 2006). It is also used, in part, when diagnosing osteoporosis in adults and children. DXA is a low-dose radiation x-ray that measures bone mineral content (BMC) and bone area (cm^2), and mathematically derived BMD (g/cm^2). The value is then compared statistically to reference populations to obtain a *Z*-score (age-matched reference) and a *T*-score ("peak" bone mass reference). Radiation exposure using DXA is low, approximately 5 to 7 μSv for a full-body scan. This allows the scan to be performed with very minimal risk (Lewis et al. 1994; Andreoli et al. 2009). Additionally, the precision of DXA is high, with coefficients of variation typically cited at around 1% (Andreoli et al. 2009).

The American College of Sports Medicine (ACSM) 2007 Female Athlete Triad position paper provides definitions for two categories used to identify low bone mass in adolescent and young adult female athletes. Both categories are defined using *Z*-scores, which are age, gender, and ethnicity-matched bone mineral density comparative values (Thompson 2004). The *Z*-score is a standard deviation score, with a value of zero indicating an average BMD compared to others in the reference database of the same age, gender, and ethnicity. A positive *Z*-score is above average, and a negative score is below average. The first category reported by the ACSM position paper is from the International Society for Clinical Densitometry (ISCD 2007). According to the ISCD (2007), a bone mass value "below the expected range for age" is defined as a BMD *Z*-score value less than or equal to –2. Because competitive athletes typically exhibit BMD *Z*-score values at or above 1, the ACSM Female Athlete Triad position paper also recognizes a *Z*-score level at or below –1 as "low bone mass for age" in female adolescent and adult competitive athletes (WHO Study Group 1994; Nattiv et al. 2007).

Even though it is less common for a female athlete to exhibit osteoporosis (compared to low bone mass), especially during adolescence or as a young adult, it is possible that an athlete may be diagnosed with this condition. There are specific criteria used to define osteoporosis in children and adults. The ISCD emphasizes that a diagnosis of osteoporosis in children and adolescents requires *both* a bone mineral density value "below the expected range for age" (≤–2) and a clinically significant fracture history (ISCD 2007). According to this definition, a clinically significant

fracture history includes one or more of the following: a previous long bone fracture of the lower extremities, a vertebral compression fracture, or two or more long bone fractures of the upper extremities (ISCD 2007). In children and adolescents, a BMD *Z*-score of less than or equal to –2 corresponds to the lowest 2.5% of an age, gender, and ethnicity-matched reference population. A child or adolescent with bone mass "below the expected range for age" but without a clinically significant fracture history does not meet the criteria for osteoporosis.

In postmenopausal women, the diagnosis of osteoporosis is determined using the *T*-score. A *T*-score is a reference value that represents an individual's bone mineral density value compared to the mean bone density of a healthy woman, age 20 to 29 years, from the NHANES III database (Lane 2006). The *T*-score was developed to quantify the risk of developing a fragility fracture in postmenopausal women age 60 years or older (Licata 2009). Therefore, the use of the *T*-score to diagnose osteoporosis is validated and, therefore, indicated primarily among postmenopausal women and men age 50 years and older. Use of the *T*-score is not indicated for children and adolescents because they have not yet reached their peak bone mass (Thompson 2004). According to the World Health Organization (WHO) and the ISCD, in postmenopausal women and men over age 50 years, osteoporosis is defined as a *T*-score value equal or less than –2.5 at the lumbar spine, total hip, femoral neck, or the 33% radius (WHO Study Group 1994).

Quantitative computed tomography (QCT) is another method used to assess bone density. This equipment measures three-dimensional, volumetric bone mineral density, expressed as grams per cm^3. It also evaluates qualitative aspects of bone, such as bone microarchitecture and bone geometry, by separately measuring cortical and trabecular bone (Boutroy et al. 2005). Therefore, a QCT scan can detect isolated change in the metabolically active trabecular bone during periods of high turnover, such as during growth in adolescence or the onset of menopause later in life. QCT can also track effects of diet, exercise, or pharmacologic treatments on cortical and trabecular bone (Lane 2006). Though the QCT scan measures more aspects of bone, it exposes an individual to higher levels of radiation, is more expensive, and the scans can be more difficult to interpret than DXA.

QCT reference data are not yet adequate to determine normative age, ethnicity, and gender-specific norms and clinical cutoffs for osteoporosis and fracture risk. Therefore, volumetric density values are not currently used in the diagnosis of osteoporosis or low bone mass (Boutroy et al. 2005). However, because fracture risk increases as a result of both bone mineral loss and microarchitecture deterioration (Boutroy et al. 2005), the development of the QCT reference database may play a critical role in screening and determining fracture risk among relevant populations.

One bone remodeling cycle lasts approximately 3 months, and as a result, significant changes in bone density take 6 to 12 months to observe. Therefore, the DXA and QCT are used to evaluate long-term changes in bone mass. However, biomarkers of bone turnover, taken from the blood, serve as indicators of short-term changes in bone metabolism. There are several biomarkers that can be measured, and each provide specific information about rate of bone formation, bone resorption, or overall bone turnover. Due to the wide variability of bone turnover markers, the use of these markers is limited to clinical research studies where statistics are used to detect

group differences rather than assess single values. Recently, newer analytical methods have reduced their variability (Civitelli et al. 2009). In addition, current research suggests that bone turnover markers may also have the ability to predict fracture risk, independent of BMD (Singer and Eyre 2008; Civitelli et al. 2009). Therefore, in the future, bone biomarkers may be useful in the monitoring of individuals' short-term response to treatment and provided as a complementary method of assessing fracture risk (Singer and Eyre 2008).

Currently, a DXA scan is not indicated as a standard of care screening tool for adolescent and young adult female athletes (Bachrach and Sills 2011). However, if a female athlete exhibits one or more risk factors, such as primary or secondary amenorrhea, current or previous eating disorder, or diagnosis of a stress fracture, a physician may recommend that the athlete undergo a DXA scan to assess her bone mineral density (Nattiv et al. 2007). The physician can use this as an additional tool to evaluate the athlete's overall health and fracture risk. This will also serve as a baseline measure of bone mass, with which future DXA scans can be compared. It is recommended that a 12-month follow-up scan be used to reevaluate athletes who continue to exhibit symptoms of the Triad (Nattiv et al. 2007).

LOW BONE MASS AND FRACTURE RISK IN SPORT

Despite the many benefits of participating in competitive sports, involvement in some sport types has been associated with a greater incidence of low BMD and higher risk of sustaining bone injuries as a result. According to the American College of Sports Medicine (Nattiv et al. 2007), sport types most at risk are those that favor or contribute to a lean build or thin frame (i.e., "thin-build" sports). These include endurance sports and sports where athletes are judged based on their appearance, require revealing uniforms, or utilize weight classes. Some of these sports include cross-country running, cross-country skiing, dance, cheer, gymnastics, ice skating, wrestling, swimming, and diving.

Several studies conducted among collegiate, postcollegiate, and adolescent female athletes reported lower bone mass in athletes who participate in thin-build sports. Beals and Hill (2006) in their study of 112 U.S. collegiate athletes found that lean-build athletes met the –1 BMD *Z*-score cutoff more than athletes in non-lean-build sports. When studying 183 female high school athletes, Barrack, Rauh, and Nichols (2010) found that endurance runners compared to athletes participating in moderate- to high-impact, non-lean-build sports exhibited a significantly higher prevalence of meeting the –1 and –2 *Z*-score criteria for low bone mass. Additionally, Cobb et al. (2003) found that approximately 40% of collegiate or postcollegiate and adolescent endurance runners, respectively, met the –1 low bone mass for age *Z*-score cutoff. This percent is higher than the approximately 16% prevalence of low bone mass expected in a normal population.

In athletes, low BMD may reduce the ability to sustain impact from exercise and, therefore, increase the risk of developing a fracture or a bone stress injury. In adult women, there is a strong relationship between bone density and fracture risk. Prior studies show that in postmenopausal women, for every 1 SD decrease in bone density *T*-score, fracture risk doubles (Jackson et al. 2008). However, prior

studies that evaluated the relationship between bone density levels and rate of bone stress injury in adolescents and young adult athletes yield conflicting results. Some research among cross-country runners, track-and-field athletes, and female adolescents report an association between lower bone mass (Bennell et al. 1996; Kelsey et al. 2007) or a family history of osteoporosis (Kelsey et al. 2007; Loud et al. 2007) and fracture risk; however, other studies report no association (Frusztajer et al. 1990; Bennell et al. 1995; Korpelainen et al. 2001; Popp et al. 2009).

Several studies that prospectively assessed female athletes report a higher risk of injury among those with lower bone mass. Bennell et al. (1996) and Kelsey et al. (2007), who followed female adult and young adult endurance runners for 1 to 2 years, found a significant relationship between lower bone mass and higher risk of developing a stress fracture during the observation period. Additionally, Rauh et al. (2010) prospectively assessed the rate of musculoskeletal injury among high school athletes participating in a variety of sport types. Among the high school athletes, low BMD (*Z*-score less than –1) was associated with a significantly increased rate of injury (including soft-tissue and bone-related injury) (Rauh et al. 2010). These studies emphasize the importance of practicing behaviors that increase bone mass, because higher bone density levels may reduce injury risk and allow for continued participation in one's sport.

It has also been suggested that bone quality may affect fracture risk (Popp et al. 2009) and that the etiology of stress fractures and other bone stress injuries is complex. Factors such as training frequency, intensity and duration, sport type, biomechanics, shoe type, and recovery have been suggested to play a role. Although lower bone density has been reported to increase the risk of sustaining a stress injury to bone, further research is necessary to identify the relationship role of bone quality and other factors that may influence fracture risk in female athletes.

Although a number of possible causes have been hypothesized, the current evidence suggests that low energy availability is a primary risk factor for low bone mass in healthy female athletes (Nattiv et al. 2007). As described in Chapter 9, low energy availability can develop inadvertently or intentionally through dietary restriction and increases in energy expenditure without a subsequent increase in intake.

Prior research studies that did not directly measure energy availability report associations between factors that either contribute to low EA (i.e., factors that decrease energy intake or increase exercise energy expenditure) or suggest low energy availability (i.e., underweight) and low bone mass in female athletes and exercising women. Examples of low EA-related factors associated with either reduced bone mass or increased bone resorption include low body weight (classification with a BMI <10% for age in an adolescent population) (Barrack, Van Loan et al. 2010), an increased training volume (among adolescent and adult endurance runners) (Hind et al. 2006; Barrack et al. 2008; Barrack, Rauh, and Nichols 2010), cognitive dietary restraint (Barrack Rauh, Barkai et al. 2008; Vescovi et al. 2008), and suppressed resting energy expenditure (De Souza et al. 2008). Functional hypothalamic amenorrhea (FHA), a condition that may result from low EA, has also been strongly associated with low bone mass among female athletes participating in a variety of sports (Gibson et al. 2004; Torstveit and Sundgot-Borgen 2005a; Beals and Hill 2006; Hind

et al. 2006; Nichols et al. 2006; Barrack, Rauh, and Nichols 2008). Therefore, these and other related factors may be used to indicate an increased risk of developing low bone mass and bone stress injury due to factors related to the Female Triad.

Regardless of the cause, low energy availability negatively impacts bone by suppressing certain key hormones (namely, leptin, estradiol, and IGF-1) that play a role in bone formation. Leptin is secreted by the adipocytes (fat cells) in the body and is considered a long-term regulator of appetite (Chan and Mantzoros 2005). It exerts both direct and indirect effects on bone metabolism. After eating, the rise in glucose and insulin stimulates the production of leptin by adipocytes. Leptin passes through the blood-brain barrier and binds to its receptor on the arcuate nucleus of the hypothalamus (Chan and Mantzoros 2005). The binding of leptin to the arcuate nucleus stimulates the synthesis of releasing hormones that initiate several hypothalamic pituitary signaling cascades, particularly those related to growth hormones, the gonadal hormones, and thyroid hormones (Chan and Mantzoros 2005). Several of these hormones, including insulin-like growth factor-1 (IGF-1) and estradiol, exert direct positive effects on bone.

Both estradiol and IGF-1 promote bone formation (Kasukawa et al. 2004; Sipos et al. 2009). Estrogen positively influences bone mass through several mechanisms. When bound to osteoblasts, estrogens upregulate production of osteoprotegerin (OPG), which reduces bone resorption. Additionally, estrogens upregulate osteoblast production of other bone-building compounds, such as IGF-1 and transforming growth factor-β (Sipos et al. 2009). Estradiol also affects bone mass by regulating gut calcium absorption and renal calcium excretion. Moreover, estradiol reduces the activity of osteoclastogenic cytokine, T cells, and other inflammatory factors (such as IL-1, IL-6, PGE_2, TNFα, MHC) that promote bone demineralization (Sipos et al. 2009). Additionally, female athletes with menstrual irregularities or low estrogen levels exhibit reduced bone mass.

In vitro, IGFs have been shown to increase the synthesis of bone matrix proteins and lower collagen deterioration (Kasukawa et al. 2004). IGF-1 has also been shown to increase transcellular calcium uptake in rats (Fleet et al. 1994). Animal and human studies support the influence of IGFs on bone mass and size. Systemic IGF-1 administration has been shown to increase cortical and trabecular bone in normal, healthy adult female rats as well as rats induced with osteopenia from gonadal hormone deficiency or unloading (Kasukawa et al. 2004). Recombinant human IGF-1 administration has also been reported to increase markers of bone deposition (PINP) in adolescents and young adult women with anorexia nervosa (Grinspoon et al. 1996; Misra et al. 2009).

Leptin acts directly at the level of the bone to promote bone formation and reduce bone resorption via its effects on osteoblasts and osteoclasts (Thomas 2004). Leptin has been shown to promote osteoblast formation, suppress osteoblast apoptosis, and indirectly lower osteoclast maturation (Thomas 2004). In animal studies, *ob/ob* leptin-deficient mice that received exogenous administration of leptin exhibited a significant increase in cortical bone accrual (Steppan et al. 2000; Thomas 2004). Furthermore, in humans, 3-month administration of recombinant human leptin to women with functional hypothalamic amenorrhea was associated with a significant increase in estradiol, IGF-1, LH, and markers

of bone formation (Welt et al. 2004). Therefore, it appears important for female athletes to consume adequate energy, particularly during periods of growth, to allow for the normal functioning of hormones that promote calcium absorption and bone formation.

FACTORS THAT INFLUENCE BONE MASS

There are a variety of factors that influence a female athlete's ability to accrue and maintain bone mass. Genetics play the largest role; however, behavioral and lifestyle factors affect the ability to reach their genetic potential. Typically among athletes, the most influential lifestyle and behavioral factors are related to nutritional intake and exercise.

Genetics

Twin and family studies underscore the influence of genetic factors on a woman's bone mineral accrual and maintenance of lifetime bone mass (Heaney et al. 2000; McGuigan et al. 2002). Reports suggest that genetics may account for 50 to 85% of the variation in bone mass, depending on the site measured (McGuigan et al. 2002). Some genes that may play a role in bone mass development are those that influence body size, hormone synthesis and secretion, and calcium absorption and utilization (Heaney et al. 2000). Vitamin D receptor (VDR) polymorphisms provide an example of one such variation, as the VDR gene polymorphisms disrupt normal vitamin D activity, alter parathyroid hormone, and lower calcium absorption and bone mass (Carling et al. 1995, 1997; Ames et al. 1999; Eisman 1999). Polymorphisms of the collagen Iα1, transforming growth factor receptor, calcitonin receptor gene are also thought to negatively affect bone (Eisman 1999). Although genetics play a large role in achieving and maintaining strong bones, environmental factors such as exercise and nutritional behaviors can either augment or detract from one's genetic potential (Eisman 1999; McGuigan et al. 2002). Therefore, genetic and environmental factors appear interrelated and together influence a female athlete's bone growth and development.

Lifestyle

There are a number of lifestyle factors that affect the accrual and maintenance of bone mass. The key factors that will be discussed in detail relate to exercise, nutrition, energy homeostasis, and hormone levels. However, other behaviors such as smoking, alcohol consumption, and use of certain medications also affect bone. In some studies, women smokers exhibit lower bone density and, among women age 60 years, continuous smokers have been reported to exhibit a 17% higher risk of hip fracture compared to nonsmokers (NIH 2000). Long-term use of steroid medications such as glucocorticoids, alcoholism, and the hormonal contraceptive Depo Provera lead to reductions in bone density (NIH 2000; Bachrach and Sills 2011). Use of additional medications, including antidepressants, inhaled steroids, chemotherapeutic drugs, vitamin A or synthetic retinoids, among others, may also negatively affect

bone. It is important for female athletes to be aware of the effect of these lifestyle factors and to limit, if medically feasible, those that negatively affect bone.

Exercise

Among female athletes, exercise facilitates the attainment and maintenance of an optimal peak BMD. There are two primary modes of exercise that exert the greatest benefit to bone among active women across the life span: high-impact, powerful movements such as jumping, side-to-side accelerations, or sprints provide intermittent, mechanical stresses to bone, and muscle fiber contractions exert smaller amplitude strains that also stimulate bone mineralization (Borer 2005). High-intensity, intermittent exercise additionally benefits bone by enhancing the secretion of hormones, such as growth hormone and IGF-I, that promote bone mineral gains (Borer 2005). Exercise also promotes mineralization by increasing the blood supply and nutrient delivery to muscles and bone (Borer 2005).

Results from prior studies suggest that a relatively small volume of loading is needed to facilitate an adaptive change to bone (Rubin and Lanyon 1984; Umemura et al. 1997; Robling et al. 2000, 2002). This is due, in part, to the fact that the cellular response of bone to a mechanical load exhibits a low saturation point (Burr et al. 2002). One study found that 36 cycles of physiologic strain magnitudes per day yielded a nondifferent bone response as compared to 1800 cycles per day of an equal magnitude strain (Rubin and Lanyon 1984). These data demonstrate that there is a threshold or saturation point above which additional strain exposures do not benefit bone. In another study among a sample of rats that participated in jumping 5 to 100 times per day for 8 weeks, rats that jumped 10 times per day did not receive more benefit than those that jumped 100 times per day (Umemura et al. 1997). These data suggest that beyond a certain number of repetitions, the bone becomes desensitized to the impact, and further benefits do not occur.

Researchers have evaluated the length of time needed to resensitize cells and allow them to respond again to exercise. Robling et al. induced 360 cycles of mediolateral bending of the right tibia each day for 5 days in four groups of rats (Robling et al. 2000). The groups were subject to either 360 cycles once/day, 180 cycles twice/day, 90 cycles four times/day, or 60 cycles six times/day, separated by 0 hours, 6 hours, 3 hours, and 2 hours, respectively (Robling et al. 2000). Rats that received 90 cycles four times/day and 60 cycles six times/day exhibited the largest osteogenic response (Robling et al. 2000). This suggests that shorter-duration high-impact loads allow for cell resensitization and promote more favorable changes to bone. Findings from these studies can inform female athletes and those who work with female athletes when designing an exercise program.

Resistance training is an exercise type that exerts low-frequency, moderate to high loading strains to bone. Previous animal and human studies document bone's adaptive response to resistance training. Westerlind et al. (1998) trained rats to perform lower-body squat-like exercise over an 8-week period. Over time, the weight that the rats lifted gradually increased. The bone of the resistance training group increased in trabecular number and trabecular thickness, and the percentage of cancellous bone increased. Additionally, the resistance training group exhibited a

higher osteocalcin mRNA level, which then leads to bone formation (Westerlind et al. 1998). In another study, Nichols et al. (2001) evaluated the effect of a 15-month resistance training intervention among a sample of 67 adolescent girls ages 14 to 17 years. Despite a high dropout rate, resistance-trained girls exhibited a significant (40%) increase in leg strength and femoral neck BMD. No strength or bone mass changes were identified among the control group in girls (Nichols et al. 2001). In another study, Wang et al. (2007) observed the relationship between maximal isometric voluntary contraction (MVC) of the left elbow flexors and knee extensors and bone mass among a sample of 258 girls ages 10 to 13 years. Maximal isometric voluntary contraction was directly correlated with BMC ($r = 0.54$ and $r = 0.50$ in the arms and legs, respectively). These findings support the beneficial effect of resistance training on the quality and quantity of bone in growing girls and support its use as a method of optimizing bone strength of active females.

Even though most sports types positively influence female athletes' bone (due to involvement of muscle contractions and, in most exercises, some degree of loading), some are more beneficial than others. Several studies among female competitive athletes report higher bone mass levels among athletes who participate in high-impact, multidirectional exercise activities compared to athletes who compete in sports that are non-weight-bearing (such as swimming, diving, and water polo) or that largely involve moderate- to low-impact, unidirectional movements (such as walking, running, and cycling) (Taaffe et al. 1995; Courteix et al. 1999; Torstveit and Sundgot-Borgen 2005b; Mudd et al. 2007). Torstveit and Sundgot-Borgen (2005a) in a study of 938 Norwegian elite athletes and 900 nonathlete controls categorized athletes into three groups (low impact, medium impact, and high impact) according to the degree of mechanical loading for each sport. From their analysis, they found that athletes participating in high-impact sports had significantly higher ($P < 0.001$) bone mineral density values at each bone site (total body, lumbar spine, total femur, femoral neck, Ward's triangle, trochanter, femoral shaft) than those participating in both low-impact and moderate-impact sports (Torstveit and Sundgot-Borgen 2005a). Examples of sports in the high-impact group included track and field sprint events, gymnastics, volleyball, power lifting, basketball, sprint speed skating, tennis, and alpine skiing. Sports in the low- and medium-impact groups included swimming, cycling, curling, race walking, middle- and long-distance running, sailing, cross-country skiing, table tennis, and wrestling.

Additional research studies in female athletes report similar results. Upon evaluation of bone mass in young adult eumenorrheic gymnasts, swimmers, and nonathlete controls, Taaffe et al. (1995) found that gymnasts had significantly higher body mass, adjusted lumbar spine, femoral neck, and trochanter BMD than swimmers and nonathlete controls. Courteix et al. (1999) also found, among prepubertal girls who had participated in at least 3 years of either swimming or gymnastics, that the gymnasts had significantly higher BMD at the radius, vertebrae, femoral neck, and Ward's triangle.

Mudd et al. (2007) in their study of 99 collegiate athletes who participated in gymnastics, softball, volleyball, cross-country, track and field, field hockey, soccer, crew, or swimming and diving found that, in their sample, runners had significantly lower bone density values at nearly all bone sites compared to athletes in all other sports. This may be due, in part, to the fact that endurance running exposes bone

to continuous, lower-impact loads rather than the intermittent, higher-impact loads exerted in other sport types. Running also involves unidirectional movement and does not stress bone from multiple directions. Competitive runners in this and other studies also reported an elevated prevalence of amenorrhea and oligomenorrhea, which, as discussed below, is also associated with low bone mass.

Nutrition

Several key nutrients play a role in the process of bone mineralization. Calcium and vitamin D are the most significant; however, many other vitamins and minerals are involved in the formation and mineralization of bone. It is recommended that female athletes consume adequate energy to allow for normal functioning of the hormones that regulate the processes of bone formation and bone resorption. Furthermore, to optimize bone mass and strength, it is advised that female athletes consume the recommended levels of each key vitamin and mineral that plays a role in bone formation. More specific information on the recommended diet for optimal bone health among female athletes is included in Chapter 6.

INTERACTION BETWEEN MENSTRUAL FUNCTION AND SPORT TYPE

Menstrual disturbances are established risk factors of low bone mass. This connection has been borne out of the research that consistently demonstrates lower bone mass levels among female athletes with functional hypothalamic amenorrhea or oligomenorrhea (Cobb et al. 2003; Gibson et al. 2004; Beals and Hill 2006; Nattiv et al. 2007; Barrack, Rauh, and Nichols 2008). Accumulating evidence indicates that inadequate energy availability precedes most if not all functional hypothalamic menstrual disturbances in otherwise healthy female athletes (Williams et al. 2001; Ihle and Loucks 2004).

However, unlike most amenorrheic athletes who exhibit reduced bone mass, amenorrheic athletes who participate in sports that involve high-impact, multidirectional activities maintain relatively high bone mineral density values. This has been demonstrated particularly among competitive gymnasts. Fehling et al. (1995) studied the bone mass values of female collegiate volleyball players, swimmers, gymnasts, and nonathlete controls. Despite a 77% prevalence of oligo- or amenorrhea, the gymnasts had a significantly higher lumbar spine, femoral neck, Ward's triangle, and total body BMD than the swimmers and nonathlete controls (Fehling et al. 1995). The gymnasts also had significantly higher lumbar spine, femoral neck, Ward's triangle, and total body BMD than the swimmers and nonathlete controls. Similarly, Robinson et al. (1995) conducted a study among a sample of collegiate gymnasts, runners, and nonathlete controls. The gymnasts and runners both had a significantly higher prevalence of amenorrhea compared to the control group. Despite the higher prevalence of amenorrhea, gymnasts exhibited a significantly higher femoral neck bone density value than controls. Gymnasts also had higher lumbar spine and total-body bone density values than runners. From these findings, it appears that the activities characteristic of gymnastics may benefit bone even among athletes with

amenorrhea. Further, they suggest that in contrast to gymnastics, the unidirectional moderate-impact, repetitive activity characteristic of sports like endurance running may not protect bone against the hormone disturbances that occur during periods of menstrual dysfunction.

RECOMMENDATIONS FOR OPTIMIZING BONE MASS

To maximize bone strength and reduce fracture risk, it is recommended that female athletes consume an adequate diet, avoid smoking and excessive alcohol intake, and participate in regular bone-building exercises. A diet consisting of adequate energy (calories), protein, and bone-building micronutrients will provide the fuel and building blocks needed to form bone. A level of energy intake that leads to an energy availability of 45 kcal/kg FFM/day will promote adequate hormone levels (Ihle and Loucks 2004). It is recommended that adolescents and adults consume 1300 mg and 1000 mg of calcium per day, respectively (Dietary Reference Intakes 1997). Vitamin D intake should meet or exceed 600 IU (up to 4000 IU) per day (Dietary Reference Intakes 1997). Female athletes who portray signs of disordered eating or an eating disorder should be screened and referred to a sports medicine physician, dietitian, and psychologist that specialize in eating disorders (Nattiv et al. 2007).

In addition, exercises that involve multiplanar, intermittent movements and elicit high-impact loads promote the greatest bone mass gains. Specific sports that integrate these type of movements include volleyball, basketball, gymnastics, tennis, soccer, and track and field (jumping and sprinting events) (Torstveit and Sundgot-Borgen 2005a). Therefore, participating in these and similar sports types will promote bone mineral gains during adolescence and optimize the maintenance of bone mass in adulthood. Resistance or strength training elicit powerful muscle contractions that strain bone and further elicit mineralization. Therefore, female athletes can supplement their training with resistance exercises to optimize benefits to bone. Strength training can be particularly beneficial for female athletes who participate in sports that elicit low- to moderate-impact forces to bone, such as cycling, swimming and diving, long-distance running, and rowing. It is recommended to lift weights two to three times per week and focus on all of the major muscle groups in the body (ACSM 2006). Functional multiplanar resistance training exercises that utilize the core, upper body, and lower body comprehensively stress bone and should be integrated into a supplementary training program. Multiplanar hops, box jumps, and training with a weight vest load bone, also promotes mineralization (Martyn-St. James and Caroll 2010). It is important for female athletes to consult with their physician before starting a new exercise program. However, if gradually introduced into a training program, these exercises can benefit female athletes' bone by increasing bone strength, reducing fracture risk, and promoting continual participation and improvement in their sport.

To optimize treatment outcomes, the American College of Sports Medicine 2007 Female Athlete Triad Position Paper recommends that athletes with low bone mass due to factors related to the Triad seek counsel from a multidisciplinary treatment team consisting of a physician, registered dietitian, and (among athletes with disordered eating) a mental health practitioner (Nattiv et al. 2007). Behavioral

interventions that aim to improve energy availability by increasing energy intake or decreasing exercise energy expenditure are recommended over pharmacologic treatment as a first line of treatment. Among amenorrheic athletes, weight gain alone has been associated with BMD gains of up to 5% per year (Lindberg et al. 1987; Zanker et al. 2004; Fredericson and Kent 2005). Research studies that assessed the effectiveness of oral contraceptive pills (OCPs) or hormone replacement therapy to increase BMD in women and athletes with functional hypothalamic amenorrhea (FHA) provide conflicting results.

CONCLUSIONS

It is important for female athletes to optimize the density and quality of bone to increase strength and reduce the risk of injury. Though some female athletes may be at risk for low bone mass, various behavioral strategies can increase bone mass and strength. These include consuming a diet sufficient in calories, protein, and bone-building nutrients and participating in moderate exercise that elicits loading to bone. It is important for health professionals to identify female athletes at risk and encourage behaviors that improve bone health. This can be promoted through advocacy and education to parents, athletes, and coaches. Through these efforts we can together work to increase the health, performance, and longevity of female athletes in sport.

REFERENCES

Ahlborg H G, Johnell O, Turner C H, Rannevik G, Karlsson M K. Bone loss and bone size after menopause. *N Engl J Med* 2003;349:327–334.

American Academy of Pediatrics, Committee on Sports Medicine. Amenorrhea in adolescent athletes. *Pediatrics* 1989 Sep;84(2):394–395.

American College of Sports Medicine, *ACSM's Guidelines for Exercise Testing and Prescription,* 7th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2006, p. 154Y8.

Ames S K, Ellis K J, Gunn S K, Copeland K C, Abrams S A. Vitamin D receptor gene Fok1 polymorphism predicts calcium absorption and bone mineral density in children. *J Bone Miner Res* 1999;14:740–746.

Andreoli A, Scalzo G, Masala S, Tarantino U, Guglielmi G. Body composition assessment by dual-energy X-ray absorptiometry (DXA). *Radiol Med* 2009;114:286–300.

Arabi A, Nabulsi M, Maalouf J, et al. Bone mineral density by age, gender, pubertal stages, and socioeconomic status in healthy Lebanese children and adolescents. *Bone* 2004;35:1169–1179.

Bachrach L K, Sills I N. Clinical report—Bone densitometry in children and adolescents. *Pediatrics* 2011;127:189–194.

Bailey D A, Martin A D, McKay H A, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: A longitudinal analysis. *J Bone Miner Res* 2000;15:2245–2250.

Barrack M T, Rauh M J, Barkai H S, Nichols J F. Dietary restraint and low bone mass in female adolescent endurance runners. *Am J Clin Nutr* 2008;87:36–43.

Barrack M T, Rauh M J, Nichols J F. Prevalence of and traits associated with low BMD among female adolescent runners. *Med Sci Sports Exerc* 2008;40:2015–2021.

Barrack M T, Rauh M J, Nichols J F. Cross-sectional evidence of suppressed bone mineral accrual among female adolescent runners. *J Bone Miner Res* 2010;25(8):1850–1857.

Barrack M T, Van Loan M D, Rauh M J, Nichols J F. Physiologic and behavioral indicators of energy deficiency in female adolescent runners with elevated bone turnover. *Am J Clin Nutr* 2010;92:652–659.

Beals K A, Hill A K. The prevalence of disordered eating, menstrual dysfunction, and low bone mineral density among U.S. collegiate athletes. *Int J Sport Nutr Exerc Metab* 2006;16:1–23.

Bennell K L, Malcolm S A, Thomas S A, et al. Risk factors for stress fractures in female track-and-field athletes: A retrospective analysis. *Clin J Sport Med* 1995;5:229–235.

Bennell K L, Malcolm S A, Thomas S A, et al. Risk factors for stress fractures in track and field athletes. A twelve-month prospective study. *Am J Sports Med* 1996;24:810–818.

Borer K T. Physical activity in the prevention and amelioration of osteoporosis in women: Interaction of mechanical, hormonal and dietary factors. *Sports Med* 2005;35:779–830.

Borer K T, Wuorinen E, Chao C, Burant C. Exercise energy expenditure is not consciously detected due to oro-gastric, not metabolic, basis of hunger sensation. *Appetite* 2005;45:177–181.

Boutroy S, Bouxsein M L, Munoz F, Delmas P D. *In vivo* assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab* 2005;90:6508–6515.

Burr D B, Robling A G, Turner C H. Effects of biomechanical stress on bones in animals. *Bone* 2002;30:781–786.

Carling T, Kindmark A, Hellman P, et al. Vitamin D receptor genotypes in primary hyperparathyroidism. *Nat Med* 1995;1:1309–1311.

Carling T, Rastad J, Kindmark A, Lundgren E, Ljunghall S, Akerstrom G. Estrogen receptor gene polymorphism in postmenopausal primary hyperparathyroidism. *Surgery* 1997;122:1101–1105; discussion 1106.

Chan J L, Mantzoros C S. Role of leptin in energy-deprivation states: Normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet* 2005;366:74–85.

Civitelli R, Armamento-Villareal R, Napoli N. Bone turnover markers: Understanding their value in clinical trials and clinical practice. *Osteoporos Int* 2009;20:843–851.

Cobb K L, Bachrach L K, Greendale G, et al. Disordered eating, menstrual irregularity, and bone mineral density in female runners. *Med Sci Sports Exerc* 2003;35:711–719.

Courteix D, Lespessailles E, Jaffre C, Obert P, Benhamou C L. Bone material acquisition and somatic development in highly trained girl gymnasts. *Acta Paediatr* 1999;88:803–808.

Datta H K, Ng W F, Walker J A, Tuck S P, Varanasi S S. The cell biology of bone metabolism. *J Clin Pathol* 2008;61:577–587.

De Souza M J, West S L, Jamal S A, Hawker G A, Gundberg C M, Williams N I. The presence of both an energy deficiency and estrogen deficiency exacerbate alterations of bone metabolism in exercising women. *Bone* 2008;43:140–148.

Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Food and Nutrition Board, Institute of Medicine, National Academy of Sciences, 1997.

Ducher G, Turner A I, Kukuljan S, Pantano K J, Carlson J L, Williams N I, De Souza M J. Obstacles in the optimization of bone health outcomes in the female athlete triad. *Sports Med* 2011;41:587–607.

Eisman J A. Genetics of osteoporosis. *Endocr Rev* 1999;20:788–804.

Fehling P C, Alekel L, Clasey J, Rector A, Stillman R J. A comparison of bone mineral densities among female athletes in impact loading and active loading sports. *Bone* 1995; 17:205–210.

Fleet J C, Bruns M E, Hock J M, Wood R J. Growth hormone and parathyroid hormone stimulate intestinal calcium absorption in aged female rats. *Endocrinology* 1994;134:1755–1760.

Fonseca A S, Szejnfeld V L, Terreri M T, Goldenberg J, Ferraz M B, Hilario M O. Bone mineral density of the lumbar spine of Brazilian children and adolescents aged 6 to 14 years. *Braz J Med Biol Res* 2001;34:347–352.

Fredericson M, Kent K. Normalization of bone density in a previously amenorrheic runner with osteoporosis. *Med Sci Sports Exerc* 2005;37:1481–1486.

Frusztajer N T, Dhuper S, Warren M P, Brooks-Gunn J, Fox R P. Nutrition and the incidence of stress fractures in ballet dancers. *Am J Clin Nutr* 1990;51:779–783.

Gibson J H, Mitchell A, Harries M G, Reeve J. Nutritional and exercise-related determinants of bone density in elite female runners. *Osteoporos Int* 2004;15:611–618.

Gordon C M, Grace E, Emans S J, et al. Effects of oral dehydroepiandrosterone on bone density in young women with anorexia nervosa: A randomized trial. *J Clin Endocrinol Metab.* 2002;87:4935–4941.

Grinspoon S, Baum H, Lee K, Anderson E, Herzog D, Klibanski A. Effects of short-term recombinant human insulin-like growth factor I administration on bone turnover in osteopenic women with anorexia nervosa. *J Clin Endocrinol Metab* 1996;81:3864–3870.

Gulekli B M, Davies M C, Jacobs, H S. Effect of treatment on established osteoporosis in young women with amenorrhoea. *Clin Endocrinol* 1994;41:275–281.

Hadjidakis D J, Androulakis, I I. Bone remodeling. *Ann NY Acad Sci* 2006;1092:385–396.

Heaney R P, Abrams S, Dawson-Hughes B, et al. Peak bone mass. *Osteoporos Int* 2000;11:985–1009.

Hind K, Truscott J G, Evans J A. Low lumbar spine bone mineral density in both male and female endurance runners. *Bone* 2006;39:880–885.

The IOC Medical Commission Working Group Women in Sport. Position stand on the female athlete triad. Available at: http://multimedia.olympic.org/pdf/en_report_917.pdf (accessed September 29, 2012).

Ihle R, Loucks A B. Dose-response relationships between energy availability and bone turnover in young exercising women. *J Bone Miner Res* 2004;19:1231–1240.

Ilich J Z, Hsieh L C, Tzagournis M A, et al. A comparison of single photon and dual X-ray absorptiometry of the forearm in children and adults. *Bone* 1994;15:187–191.

International Society for Clinical Densitometry (ISCD) ISfCD. Official Positions and Pediatric Official Positions. 2007.

Jackson R D, Donepudi S, Mysiw W J. Epidemiology of fracture risk in the Women's Health Initiative. *Curr Osteoporos Rep* 2008;6:155–161.

Kasukawa Y, Miyakoshi N, Mohan S. The anabolic effects of GH/IGF system on bone. *Curr Pharm Des* 2004;10:2577–2592.

Kelsey J L, Bachrach L K, Procter-Gray E, et al. Risk factors for stress fracture among young female cross-country runners. *Med Sci Sports Exerc* 2007;39:1457–1463.

Korpelainen R, Orava S, Karpakka J, Siira P, Hulkko A. Risk factors for recurrent stress fractures in athletes. *Am J Sports Med* 2001;29:304–310.

Lane N E. Epidemiology, etiology, and diagnosis of osteoporosis. *Am J Obstet Gynecol* 2006;194:S3–S11.

Langton D M, Njeh C F (Eds). *The Physical Measurement of Bone*. Bristol and Philadelphia: Institute of Physics, 2003.

Lewis M K, Blake G M, Fogelman I. Patient dose in dual x-ray absorptiometry. *Osteoporos Int* 1994;4:11–15.

Licata A. Bone density vs bone quality: What's a clinician to do? *Cleve Clin J Med* 2009;76:331–336.

Lindberg J S, Powell M M, Hunt D E, et al. Increased vertebral bone mineral in response to reduced exercise in amenorrheic runners. *West J Med* 1987;146:39–42.

Loud K J, Gordon C M. Adolescent bone health. *Arch Pediatr Adolesc Med* 2006;160:1026–1032.

Loud K J, Micheli L J, Bristol S, Austin S B, Gordon C M. Family history predicts stress fracture in active female adolescents. *Pediatrics* 2007;120:e364–e372.

MacKelvie K J, Khan K M, McKay H A. Is there a critical period for bone response to weight-bearing exercise in children and adolescents? A systematic review. *Br J Sports Med* 2002;36:250–257; discussion 257.

Martyn-St. James M, Carroll S. Effects of different impact exercise modalities on bone mineral density in premenopausal women: A meta-analysis. *J Bone Miner Metab* 2010;28:251–267.

Marques de Aquino T, Avesani C M, Brasileiro R S, de Abreu Carvalhaes J T. Resting energy expenditure of children and adolescents undergoing hemodialysis. *J Ren Nutr* 2008;18:312–319.

McGuigan F E, Murray L, Gallagher A, et al. Genetic and environmental determinants of peak bone mass in young men and women. *J Bone Miner Res* 2002;17:1273–1279.

Meyer F, O'Connor H, Shirreffs S M. Nutrition for the young athlete. *J Sports Sci* 2007;25 Suppl 1:S73–S82.

Misra M, McGrane J, Miller K K, et al. Effects of rhIGF-1 administration on surrogate markers of bone turnover in adolescents with anorexia nervosa. *Bone* 2009;45:493–498.

Mudd L M, Fornetti W, Pivarnik J M. Bone mineral density in collegiate female athletes: Comparisons among sports. *J Athl Train* 2007;42:403–408.

Nattiv A, Loucks A B, Manore M M, Sanborn C F, Sundgot-Borgen J, Warren M P. American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc* 2007;39:1867–1882.

Nichols J F, Rauh M J, Lawson M J, Ji M, Barkai H S. Prevalence of the female athlete triad syndrome among high school athletes. *Arch Pediatr Adolesc Med* 2006;160:137–142.

Nichols D L, Sanborn C F, Love A M. Resistance training and bone mineral density in adolescent females. *J Pediatr* 2001;139:494–500.

NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, March 7–29, 2000: Highlights of the conference. *South Med J* 2001;94:569–573.

Popp K L, Hughes J M, Smock A J, et al. Bone geometry, strength, and muscle size in runners with a history of stress fracture. *Med Sci Sports Exerc* 2009;41:2145–2150.

Porterfield S P. *Endocrine Physiology*, 2nd Edition. St. Louis: Mosby, 2001.

Rauh M J, Nichols J F, Barrack M T. Relationships among injury and disordered eating, menstrual dysfunction, and low bone mineral density in high school athletes: A prospective study. *J Athl Train* 2010;45:243–252.

Rey P S-R E, Garnero P, Vey-Marty B, Delmas P D, et al. Measurement of bone density in the wrist using X-ray absorptiometry: Comparison with measurements of other sites. *Revue du rhumatisme* 1994;61:619–626.

Rickenlund A K, Carlstrom B, Ekblom T B, et al. Effects of oral contraceptives on body composition and physical performance in female athletes. *J Clin Endocrinol Metab* 2004;89:4364–4370.

Robinson T L, Snow-Harter C, Taaffe D R, et al. Gymnasts exhibit higher bone mass than runners despite similar prevalence of amenorrhea and oligomenorrhea. *J Bone Miner Res* 1995;10:26–35.

Robling A G, Burr D B, Turner C H. Partitioning a daily mechanical stimulus into discrete loading bouts improves the osteogenic response to loading. *J Bone Miner Res* 2000;15:1596–1602.

Robling A G, Hinant F M, Burr D B, Turner C H. Improved bone structure and strength after long-term mechanical loading is greatest if loading is separated into short bouts. *J Bone Miner Res* 2002;17:1545–1554.

Rubin C T, Lanyon L E. Regulation of bone formation by applied dynamic loads. *J Bone Joint Surg Am* 1984;66:397–402.

Sabatier J P, Guaydier-Souquieres G, Benmalek A, Marcelli C. Evolution of lumbar bone mineral content during adolescence and adulthood: A longitudinal study in 395 healthy females 10–24 years of age and 206 premenopausal women. *Osteoporos Int* 1999;9:476–482.

Singer F R, Eyre D R. Using biochemical markers of bone turnover in clinical practice. *Cleve Clin J Med* 2008;75:739–750.

Sipos W, Pietschmann P, Rauner M, Kerschan-Schindl K, Patsch J. Pathophysiology of osteoporosis. *Wien Med Wochenschr* 2009;159:230–234.

Steppan C M, Crawford D T, Chidsey-Frink K L, Ke H, Swick A G. Leptin is a potent stimulator of bone growth in ob/ob mice. *Regul Pept* 2000;92:73–78.

Stipanuk M H. *Biochemical and Physiological Aspects of Human Nutrition*, 1st edition. Philadelphia: W.B. Saunders, 2000.

Taaffe D R, Snow-Harter C, Connolly D A, Robinson T L, Brown M D, Marcus R. Differential effects of swimming versus weight-bearing activity on bone mineral status of eumenorrheic athletes. *J Bone Miner Res* 1995;10:586–593.

Theintz G E. Endocrine adaptation to intensive physical training during growth. *Clin Endocrinol (Oxf)* 1994;41:267–272.

Theintz G, Buchs B, Rizzoli R, et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: Evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. *J Clin Endocrinol Metab* 1992;75:1060–1065.

Thomas T. The complex effects of leptin on bone metabolism through multiple pathways. *Curr Opin Pharmacol* 2004;4:295–300.

Thompson T G. Bone Health and Osteoporosis: A Report of the Surgeon General. 2004.

Torstveit M K, Sundgot-Borgen J. Low bone mineral density is two to three times more prevalent in non-athletic premenopausal women than in elite athletes: A comprehensive controlled study. *Br J Sports Med* 2005a;39:282–287; discussion 282–287.

Torstveit M K, Sundgot-Borgen J. Participation in leanness sports but not training volume is associated with menstrual dysfunction: A national survey of 1276 elite athletes and controls. *Br J Sports Med* 2005b;39:141–147.

Umemura Y, Ishiko T, Yamauchi T, Kurono M, Mashiko S. Five jumps per day increase bone mass and breaking force in rats. *J Bone Miner Res* 1997;12:1480–1485.

Vescovi J D, Scheid J L, Hontscharuk R, De Souza M J. Cognitive dietary restraint: Impact on bone, menstrual and metabolic status in young women. *Physiol Behav* 2008;95:48–55.

Wang Q, Alen M, Nicholson P, et al. Weight-bearing, muscle loading and bone mineral accrual in pubertal girls— A 2-year longitudinal study. *Bone* 2007;40:1196–1202.

Weaver C M. Adolescence: The period of dramatic bone growth. *Endocrine* 2002;17:43–48.

Welt C K, Chan J L, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 2004;351:987–997.

Westerlind K C, Fluckey J D, Gordon S E, Kraemer W J, Farrell P A, Turner R T. Effect of resistance exercise training on cortical and cancellous bone in mature male rats. *J Appl Physiol* 1998;84:459–464.

WHO Study Group. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Report of a WHO Study Group. *World Health Organ Tech Rep Ser*, 1994:1–129.

Williams N I, Caston-Balderrama A L, Helmreich D L, Parfitt D B, Nosbisch C, Cameron J L. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: Abrupt transition to exercise-induced amenorrhea. *Endocrinology* 2001;142:2381–2389.

Zanker C L, Cooke J G, Truscott B, et al. Annual changes of bone density over 12 years in an amenorrheic athlete. *Med Sci Sports Exerc* 2004;36:137–142.

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