11 Exercise Training in the Normal Female: Effects of Low Energy Availability on Reproductive Function

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Abbreviations

ACSM	American College of Sports Medicine
BMI	Body mass index
BW	Body weight
EA	Energy availability
EI	Energy intake
FFM	Fat-free mass
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
HPG	Hypothalamic-pituitary-gonadal
IGFBP	IGF-binding protein
IGF-I	Insulin-like growth factor-I
kcal	Kilocalories
LBM	Lean body mass
LH	Luteinizing hormone
NEB	Negative energy balance
NEEE	Non-exercise energy expenditure
PYY	Peptide YY
RM	Resting metabolism
T3	Tri-iodothyronine
TEEE	Total energy expended during exercise
WEE	Waking energy expenditure

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Introduction: The Female Athlete Triad

This chapter summarizes the studies in our laboratory and others that identified low energy availability as the key factor causing the Female Athlete Triad and identifies four distinct origins of low energy availability among female athletes. In 2007, the American College of Sports Medicine (ACSM) published a revised position stand on the Female Athlete Triad [1], which replaced its earlier position stand on the same subject [2]. The revised position stand corrected the former misunderstanding of the Triad as a narrow syndrome consisting of disordered eating, amenorrhea, and osteoporosis by describing the Triad more broadly as the harmful effects of low energy availability on menstrual function and bone mineral density. The revised position stand emphasized that energy availability can be severely reduced by exercise energy expenditure alone without clinical eating disorders, disordered eating, or even dietary restriction. It also explained that low energy availability induces more menstrual disorders than amenorrhea and that these functional hypothalamic menstrual disorders must be carefully distinguished by differential diagnosis from other kinds of menstrual disorders not caused by low energy availability that are, therefore, unrelated to the Triad. The revised position stand also explained that bone mineral density in young athletes must be quantified in terms of Z-scores

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instead of T-scores and that during adolescence low energy availability can cause Z-scores to decline as T-scores increase. Subsequently, treatment and return to play guidelines for the Triad were published in 2014 by the Female Athlete Triad Coalition [3, 4]. In 2014, an International Olympic Committee consensus statement introduced the term Relative Energy Deficiency in Sport to extend the concept of the Female Athlete Triad to include effects of energy deficiency beyond the reproductive and skeletal systems in men as well as women [5]. This chapter focuses on effects of low energy availability on reproductive function, specifically in women.

Hypothetical Mechanisms of Functional Hypothalamic Menstrual Disorders in Exercising Women

As in other fields of research, competing schools of thought developed to explain the high prevalence of menstrual disorders observed in exercising women. Of the several early mechanisms proposed, three were most widely held.

Body Composition

In 1974, body composition was offered as an explanation for the amenorrhea observed in anorexia nervosa patients [6]. This idea was a refinement of an earlier hypothesis about body weight accounting for the timing of menarche [7]. The body composition hypothesis held that menarche occurs in girls when the amount of energy stored in their bodies as fat rises to a critical 17% of their body weight, and that menstrual function is lost later when their body fat declines to less than a critical 22% of body weight [6].

The body composition hypothesis was the most widely publicized explanation for menstrual disorders in athletes in the lay community and the most widely embraced by the clinical community, even though it was the least widely accepted within the scientific community. The hypothesis was based entirely on correlations without any supporting experimental evidence [8]. Actually, observations of athletes did not consistently verify an association of menstrual status with body composition (e.g., Ref. [9]) and did not display the correct temporal relationship between changes in body composition and menstrual function (for reviews, see [10–13]). Rather, eumenorrheic and amenorrheic athletes were found to span a common range of body composition [14] leaner than that of eumenorrheic sedentary women. In addition, after the growth and sexual development of prepubertal animals had been blocked by dietary restriction, normal luteinizing hormone (LH) pulsatility resumed only a few hours after ad libitum feeding was permitted, before any change in body weight or composition could occur [15]. Moreover, when surgical reduction of the stomachs of severely obese women (body weight ~130 kg; body mass index [BMI] ~47) reduced the amount of food that they could eat, rapid weight loss and amenorrhea occurred while the patients were still obese (body weight ~97 kg; BMI ~35) [16].

Despite such criticisms, scientific interest in the body composition hypothesis was renewed with the discovery in 1994 of the adipocyte hormone leptin [17], with the observation of statistically significant correlations between leptin levels and body fatness in rodents and humans (e.g., Ref. [18]) and with the discovery of leptin receptors on hypothalamic neurons. Since then, an abundance of experimental evidence from rodents and human has demonstrated that a minimal level of leptin is permissive (i.e., necessary but not sufficient) for sexual development and function [19]. This permissive effect occurs indirectly via receptors on hypothalamic kisspeptin neurons that communicate with the hypothalamic gonadotropin-releasing hormone (GnRH) neurons that regulate LH pulsatility [20].

A 9-month double-blind, randomized, clinical trial administered pharmacological doses of leptin to women with functional hypothalamic amenorrhea whose BMI was in the range 18–25 kg/m^2 [21]. Prior to treatment, their leptin levels (mean ± SD = 4.6 ± 2.0 ng/ml) were within the lower portion of the range (7.4 ± 3.7 ng/ml) cited by the leptin assay manufacturer (Millipore Corp.) for women in this range of BMI [22]. Leptin levels comparable to those reported by the manufacturer

have been found in other women with similar ranges of BMI [23–29]. The leptin dosages administered to the women with functional hypothalamic amenorrhea in this experiment raised their leptin levels more than tenfold (mean ± SD = 59 ± 37 ng/ml). Yet menstrual cycles occurred only intermittently, with the number of menstruating women fluctuating from month to month between 3 of 10 (30%) and 4 of 7 (57%).

By contrast, nutritional counseling has restored spontaneous menstrual cycles in 75% of women with functional hypothalamic amenorrhea within 5 months [30]. Although leptin was originally thought to communicate information about fat stores, it was later found to vary profoundly in response to fasting, dietary restriction, refeeding after dietary restriction, and overfeeding before any changes in adiposity occurred [31–34]. 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 [35]. In eumenorrheic and amenorrheic athletes, leptin was found to differ not in its average concentration, but rather in the presence and absence, respectively, of a diurnal rhythm [23], and the diurnal rhythm was found to depend not on energy intake but rather on energy availability or more specifically on carbohydrate availability [27]. Thus, if leptin does participate in the functional regulation of the GnRH pulse generator in exercising women, it seems more likely to do so as a signal of low energy or carbohydrate availability than as a signal of low energy stores.

Energy Availability

In 1980, Warren was the first to suggest that menstrual function in dancers might be disrupted by an "energy drain" [36], but an empirically testable energy availability hypothesis was first clearly stated in terms of brain energy availability by Winterer, Cutler, and Loriaux in 1984 [37]. They hypothesized that failure to provide sufficient metabolic fuels to meet the energy requirements of the brain causes an alteration in brain function that disrupts the GnRH pulse generator, although the mechanism of this alteration was unknown.

At the organismal level, the energy availability hypothesis recognizes that mammals partition energy among several major metabolic activities, including cellular maintenance, immunity, thermoregulation, locomotion, growth, and reproduction [38] and that the expenditure of energy in one of these functions, such as locomotion, makes it unavailable for others, such as reproduction. Considerable observational data from biological field trials supports this idea and indicated that the dependence of reproductive function on energy availability operates principally in females (For reviews, see [38–42]. Experiments had induced anestrus in Syrian hamsters by food restriction, by the administration of pharmacological blockers of carbohydrate and fat metabolism, by insulin administration (which shunts metabolic fuels into storage), and by cold exposure (which consumes metabolic fuels in thermogenesis) [38]. Disruptions of reproductive function were independent of body size and composition.

The energy availability hypothesis was also supported by endocrine observations of athletes. Amenorrheic athletes displayed low blood glucose levels during the feeding phase of the day [43], low insulin and high IGF binding protein-1 (IGFBP) during the fasting phase [43], loss of the leptin diurnal rhythm [23], high fasting acylated ghrelin [44], high peptide YY (PYY) [45], and low tri-iodothyronine (T_3) levels in the morning [46, 47]. All of these abnormalities in metabolic substrates and hormones are signs of energy deficiency. T_3 regulates basal metabolic rate, and low T_3 occurs in numerous conditions, from fasting to cancer, in which dietary energy intake is insufficient to meet metabolic demands. In addition, eumenorrheic and amenorrheic athletes both displayed low insulin and high IGFBP-1 levels during the feeding phase of the day, as well as low leptin [23] and elevated growth hormone (GH) levels over 24 hours [43]. Indeed, eumenorrheic and amenorrheic athletes were found to be distinguished not by different 24-hour mean concentrations of leptin but rather by different amplitudes in the diurnal rhythm of leptin [23].

Amenorrheic and eumenorrheic athletes reported similar stable body weights, despite dietary energy intakes similar to those of sedentary women [46, 48–52]. That is, they reported their dietary energy intakes to be much less than would be expected for an athlete's level of physical activity. This apparent discrepancy between stable body weight and unexpectedly low dietary energy intake was controversial. Since energy intake and expenditure are very difficult to measure accurately, the apparent discrepancy might have been attributable to methodological errors. Some investigators attributed the apparent discrepancy between energy intake and expenditure in athletic women to underreporting of dietary intake [53, 54], because such underreporting is common in all populations [55], but underreporting did not account for the abnormalities in metabolic substrates and hormones observed in athletes. Furthermore, behavior modification and endocrine-mediated alterations of resting metabolic rate operate to stabilize body weight despite dietary energy excess and deficiency [56].

Exercise Stress

The exercise stress hypothesis held that exercise disrupts the GnRH pulse generator by activating the hypothalamic–pituitary–adrenal axis. In order for the stress hypothesis to be meaningfully independent of the energy availability hypothesis, however, the adrenal axis must be activated independently of the energy cost of the exercise.

Certainly, there are central and peripheral mechanisms by which the adrenal axis can disrupt the ovarian axis [57], and prolonged aerobic exercise without glucose supplementation does activate the adrenal axis. Selye first induced anestrus and ovarian atrophy in rats by abruptly forcing them to run strenuously for prolonged periods [58]. Later, others also induced anestrus by forced swimming [59, 60], by forced running [61], and by requiring animals to run farther and farther for smaller and smaller food rewards [62, 63]. The elevated cortisol levels induced in such experiments were interpreted as signs of stress, and the resulting disruptions of the hypothalamic-pituitary-gonadal (HPG) axis were widely interpreted as evidence that "exercise stress" has a counter-regulatory influence on the female reproductive system.

Amenorrheic athletes also display mildly elevated cortisol levels [43, 48, 64–66]. This observation was the basis for attributing their amenorrhea to stress. Mild hypercortisolism is also associated with amenorrhea in patients with functional hypothalamic amenorrhea [67] and anorexia nervosa [68]. This interpretation overlooked the glucoregulatory functions of cortisol, which inhibit skeletal muscle glucose uptake and promote skeletal muscle proteolysis for hepatic gluconeogenesis in response to low blood glucose levels [69]. Thus, it was possible that the mild hypercortisolism observed in amenorrheic athletes might have reflected a chronic energy deficiency rather than exercise stress.

At the time, it was not known whether the adrenal cortical axis mechanisms that disrupt the HPG axis in forced exercise experiments on animals also operate in voluntarily exercising women. Indeed, up to that time, all animal experiments investigating the influence of the "activity stress paradigm" on reproductive function had confounded the stress of exercise with the stress of the method used to force animals to exercise. These experiments had also been confounded by the energy cost of the exercise performed, and glucose supplementation during exercise was found to blunt the usual rise in cortisol in both rats [70] and men [71]. As a result, in 1990 the literature on stress contained only ambiguous evidence that the stress of exercise disrupts the HPG axis in either animals or humans.

Prospective Clinical Experiments

Experiments Confounding Exercise Stress and Energy Availability

Several investigators attempted to induce menstrual disorders through chronic exercise training, but most [72–75] applied only a moderate volume of exercise, or the volume of exercise was increased gradually over several months, and

diet was uncontrolled or unquantified. One study [75] selected physically trained subjects who appeared to have been luteally suppressed before the study even began [76].

Only one experiment had successfully induced menstrual disorders in regularly menstruating women [77]. Modeled on Selye's early animal experiments [58], this single successful experiment imposed a high volume of aerobic exercise abruptly, thereby suppressing follicular development, the LH surge, and luteal function in a large proportion of the subjects in the first month and in an even larger proportion in the second. Both proportions were greater in a subgroup fed a controlled weight loss diet than in another subgroup fed for weight maintenance, but even the weight maintenance subgroup may have been underfed, since behavior modification and endocrine-mediated alterations of resting metabolic rate operate to stabilize body weight despite dietary energy excess and deficiency [56].

Such experiments, in which outcome variables are properties of the menstrual cycle, require sustained observations over a period of several weeks. Such prolonged experimental protocols suffer from practical problems with subject retention and compliance with experimental treatments. To avoid these difficulties, short-term experimental protocols were developed in which LH pulsatility was chosen as the outcome variable, because ovarian function is critically dependent on LH pulsatility. Of course, short-term effects on LH pulsatility are not proof of chronic effects on ovarian function, but hypotheses about mechanisms regulating LH pulsatility could be tested in highly controlled short-term experiments, and then chronic effects could be confirmed in prolonged experiments later.

One such short-term experimental protocol found that a combination of increased exercise and dietary restriction disrupts LH pulsatility during the early follicular phase [78]. LH pulse frequency during 12 waking hours was lower in four habitually physically active women when their exercise training regimen was increased during a few days of dietary restriction than during dietary supplementation. However, this experiment did not determine whether LH pulse frequency could be suppressed by exercise without dietary restriction or whether the stress of exercise had a suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability.

Experiments Distinguishing the Independent Effects of Exercise Stress and Energy Availability

For several years, we focused our efforts on a series of studies that we called the "Excalibur" experiments that were designed to determine the independent effects of exercise stress and energy availability on the HPG axis [28, 29, 79–83]. For these experiments, we defined energy availability operationally as dietary energy intake minus exercise energy expenditure. Conceptually, this corresponds to the amount of dietary energy remaining after exercise training for all other physiological functions. Although not the actual physiological quantity hypothetically affecting the HPG axis at the cellular level, our operational definition in behavioral terms had the advantage of being readily measurable and controllable. We controlled the dietary energy intake of our subjects by feeding them diets of known amount and composition as their only food during the experiments. We also required them to exercise under supervision in our laboratory on a treadmill while we measured and controlled their energy expenditure until they had expended a predetermined amount of energy. In the absence of any empirically operational definition of stress [84], we defined exercise stress independently as everything associated with exercise except its energy cost.

Through careful subject selection, we took steps to minimize the influence of potentially confounding factors. Healthy, regularly menstruating, habitually sedentary, nonobese, nonsmoking women 18–34 years of age at least 5 years past menarche, with no recent history of dieting, weight loss, or aerobic training were recruited. Before being admitted to the study, these volunteers underwent an extensive screening procedure, including written medical, men-

strual, dietary, and athletic histories, a physical examination, a 12-lead resting electrocardiogram, a 7-day prospective dietary record, determination of body composition by hydrostatic weighing or whole body air-displacement plethysmography, and a treadmill test to determine their aerobic capacity. Volunteers were admitted into experiments only if they presented no current use of medications including oral contraceptives and no history of heart, liver, or renal disease, diabetes, and menstrual or thyroid disorders. They must also have had documented prospective records of menstrual cycles 26–32 days in length for at least the previous 3 months. They were required to be 18–30% body fat, with habitual energy intakes between 35 and 55 kcal/kg lean body mass (LBM)/day based on their 7-day diet records, with maximal aerobic capacities less than 42 ml O_2/kg body weight (BW)/min, and they must have been performing less than 60 minutes of habitual aerobic activity per week for the previous 3 months.

The narrow range of our subjects' menstrual cycle lengths implied that we restricted our subject pool to the central 60% of menstrual cycle lengths in the population and that from this pool we chose women whose menstrual cycle lengths were in the least variable 20% of the population [85]. Thus, if anything, our subjects' reproductive systems were robust against disturbance by commonly occurring environmental and behavioral influences. We could be confident, therefore, that if our treatments disrupted the reproductive systems of these women, they would disrupt the reproductive systems of other women, too. We could also be confident that our subjects' metabolism had not been disturbed by any confounding medical conditions or dietary or exercise habits before our treatments were applied.

Excalibur I

Excalibur I [79] was designed to investigate whether exercise stress had any suppressive effect on T_3 levels independent of the impact of the energy cost of exercise on energy availability. We were interested in T_3 because it regulates the rate of energy expenditure at rest and because it was known to be suppressed in amenorrheic athletes. We reasoned that if the energy cost of exercise necessitates such major metabolic adjustments as the suppression of reproductive function, then these metabolic adjustments might be mediated in part by suppressing T_3.

Over the course of the Excalibur experiments, our insight into how to correctly quantify energy availability (EA) for subjects of various body sizes gradually matured. At the time of Excalibur I, we normalized energy intake (EI) and exercise energy expenditure to body weight (BW). We also measured exercise energy expenditure as the total energy expended during exercise (TEEE), as measured by an ergometer.

$$\mathrm{EA} = \left(\mathrm{EI} - \mathrm{TEEE}\right) / \mathrm{BW}$$

In Excalibur I, we found that severely low energy availability (8 kilocalories per kilogram of body weight per day, kcal/kgBW/day) suppressed T_3 levels by 15%, while exercise stress had no effect on T_3. T_3 levels were suppressed similarly regardless of whether energy availability was reduced by dietary energy restriction or by exercise energy expenditure. Furthermore, the suppression of T_3 in exercising women was prevented by supplementing their diet in compensation for the energy cost of their exercise. These findings were consistent with the energy availability hypothesis and inconsistent with the exercise stress hypothesis.

Excalibur II

Excalibur II [80] was designed to reveal whether T_3 levels in exercising women vary in linear proportion to energy availability or are suppressed abruptly at a particular threshold of energy availability. By this time we had realized that very little energy expenditure occurs in body fat. Accordingly, we changed our normalization of energy intake and expenditure to lean body mass (LBM) which would exclude body fat.

$$\mathrm{EA} = \left(\mathrm{EI} - \mathrm{TEEE}\right) / \mathrm{LBM}$$

We administered various levels of energy availability to exercising women and found that the suppression of T_3 by low energy availability occurred

abruptly at a threshold of energy availability near 25 kcal/kgLBM/day. For our women of average body size (59 kg) and composition (24.5% body fat), that threshold was about 1000 kcal/day.

Excalibur III

Normal ovarian function depends not on some stable concentration of LH but rather on the occurrence of pulsatile surges of LH concentrations in the blood at regular intervals. These pulses correspond to regular secretory bursts of LH from the pituitary gland in response to similar secretory bursts of GnRH from the hypothalamus. The frequency (at intervals of 70–180 minutes) and amplitude of these pulses vary around the menstrual cycle. In sedentary women in the early follicular phase, the pulsatile pattern is characterized as high frequency and low amplitude. In regularly menstruating athletes, the pulses occur less often and are larger in amplitude but still at regular intervals. In amenorrheic athletes, LH pulses occur even less often and irregularly [48].

Therefore, in Excalibur III [81, 82], we investigated whether exercise has any suppressive effect on LH pulsatility beyond the impact of its energy cost on energy availability. The design of Excalibur III is illustrated in Fig. 11.1. For 4 days in the mid-follicular phase of two menstrual cycles, we controlled the energy availability of two groups of women. During one cycle, we administered a balanced energy availability of 45 kcal/kgLBM/day, and during the other cycle, we administered a low energy availability of 10 kcal/kgLBM/day. One group of subjects performed no exercise during the two treatment periods. A second group performed the same large volume of high-intensity exercise that we had utilized in Excalibur I (30 kcal/kgLBM/day at 70% VO_2max; maximal aerobic capacity]). We imposed balanced and low energy availabilities on the non-exercising group by feeding them 45 and 10 kcal/kgLBM/day, respectively. We imposed the same balanced and low energy availabilities on the group performing 30 kcal/kgLBM/day of exercise by feeding them 75 and 40 kcal/kgLBM/day, respectively.

Between Excalibur II and III, we had had another insight into the proper quantification of energy availability. Prior to Excalibur III [81, 82],

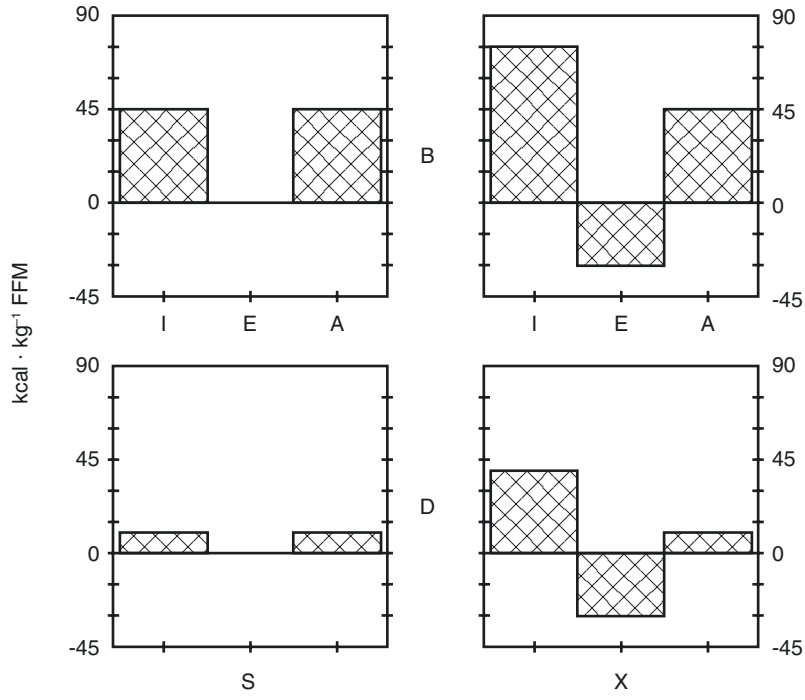

Fig. 11.1 Experimental design of Excalibur III. Dietary energy intake (I) and exercise energy expenditure (E) were controlled to achieve balanced (B = 45 kcal/kgLBM/day) and deprived (D = 10 kcal/kgLBM/day) energy availability (A = I-E) treatments. Deprived energy availability was achieved by dietary restriction alone in sedentary women (S) and by exercise energy expenditure alone in exercising women (X) (1 kcal = 4.18 kJ). (Reproduced with permission from [82])

we had calculated energy availability by subtracting total energy expenditure during exercise (TEEE) from dietary energy intake. While we were designing Excalibur III, however, we recognized that if our exercising subjects had not been exercising, their other routine activities during the same hours would have resulted in some non-exercise energy expenditure (NEEE). Therefore, the actual energy expenditure due to exercise itself (EEE) was less than the total energy expenditure measured during exercise (EEE = TEEE – NEEE). This adjustment would be especially important for Excalibur III, in which some subjects exercised and others did not. So, in Excalibur III and our later experiments, we changed again the way we calculated energy availability by subtracting from dietary energy intake only the portion of total energy expenditure during exercise that was directly attributable to the exercise itself.

$$EA = (EI - EEE) / LBM$$

We achieved this by using an activity monitor to measure our subjects' energy expenditure in their normal daily activities during the same hours of the day when they would be exercising in our experiment. We then subtracted this non-exercise energy expenditure in routine activities from their total energy expenditure during exercise to obtain the amount of energy expenditure during exercise that was specifically attributable to the exercise. In retrospect, our subjects' energy expenditure in routine activities on a non-exercising day during the same 3 hours when they exercised in Excalibur II had amounted to 5 kcal/kgLBM/day, and our calculations of energy availability had been underestimated by the same amount.

At the end of each of the 4-day treatments in Excalibur III, we admitted the women to a general clinical research center and drew blood samples from them at 10-minute intervals for 24 hours. Later, we measured the amount of LH in each sample and used a special statistical computer program to detect and to calculate the frequency and amplitude of their LH pulses. We determined the effects of energy availability on these frequencies and amplitudes by contrasting data taken while performing the same exercise at different energy availabilities, and we determined the independent effect of exercise stress by contrasting groups exercising differently at the same energy availabilities.

We found that low energy availability reduced LH pulse frequency and increased LH pulse amplitude, while exercise stress had no suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability (Fig. 11.2). LH pulsatility was disrupted by extreme energy restriction alone and by extreme

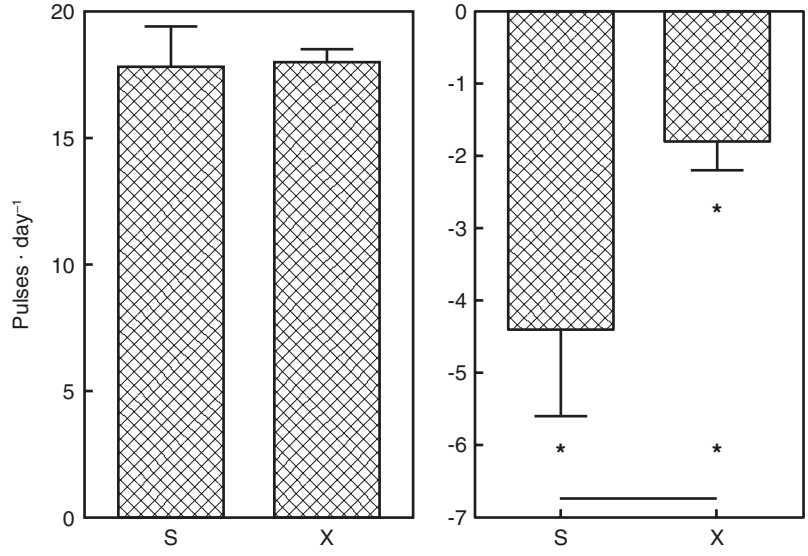

Fig. 11.2 Effects of low energy availability on LH pulsatility in Excalibur III. Left: Luteinizing hormone (LH) pulse frequency in sedentary (S) and exercising (X) women with the same balanced energy availability (45 kcal/kgLBM/day). Right: Reduction in LH pulse frequency caused by low energy availability (10 kcal/kgLBM/day) in sedentary (S) and exercising (X) women. *$p < 0.01$. (Adapted from [81, 82])

exercise energy expenditure alone. Dietary supplementation prevented the suppression of LH pulsatility by exercise energy expenditure. Others have shown that short-term fasting also reduces LH pulse frequency in sedentary women during the early follicular phase [86, 87] and that in lean women, ovarian function is also impaired during the ensuing menstrual cycle [87].

In Excalibur III, low energy availability also suppressed plasma glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, and T_3 while raising growth hormone (GH) and cortisol levels. All these effects are reminiscent of abnormalities observed in amenorrheic athletes [43, 46–48, 64–66].

This contradiction of the exercise stress hypothesis has been confirmed by more prolonged experiments on animals. 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 [88]. Then their menstrual cycles were restored by supplementing their diets without any moderation of their exercise regimen [89]. The exercise stress hypothesis was also contradicted in a novel animal model of the entire Female Athlete Triad [90]. In this modified activity stress paradigm, rats were habituated to voluntary wheel running for 90 days and then randomized to control and restricted diets for the next 90 days. Although both groups ran similar distances and expended similar amounts of energy in exercise, 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.

The suppression of LH pulse frequency by low energy availability in Excalibur III was actually *smaller* in exercising women than in non-exercising women with the same low energy availability [82]. This result was unexpected, and it suggested that LH pulsatility might actually depend on a more specific metabolic factor that is easily confused with energy availability, but which is less compromised by exercise energy expenditure than by dietary energy restriction.

Research in other mammals suggests that GnRH neuron activity and LH pulsatility are actually regulated by brain glucose availability [38, 41]. The adult female human brain oxidizes about 80 g of glucose each day at a continuous rate. This must be provided daily by dietary carbohydrate, because the brain's rate of energy expenditure can deplete liver glycogen stores in less than a day [91]. To that end, moderate exercise oxidizes as much glucose in an hour.

In the non-exercising women in Excalibur III, low energy availability due to dietary energy restriction reduced carbohydrate intake by 77%. This reduction in carbohydrate intake was similar to the 73% increase in carbohydrate oxidation revealed by respiratory gas analysis in the exercising women during the balanced energy availability treatment. By contrast, carbohydrate oxidation increased only 49% in the exercising women under low energy availability conditions. This alteration in fuel selection conserved almost 70% of the brain's daily glucose requirement. Thus, exercise may compromise brain glucose availability less than dietary energy restriction, and this may account for the smaller disruption of LH pulsatility that we observed in exercising women than in dietary-restricted women. Thus, LH pulsatility may depend specifically on carbohydrate availability rather than energy availability in women, just as it does in other mammals.

Excalibur IV

Excalibur IV [83] was designed to reveal whether refeeding reverses the suppression of LH pulsatility in women as quickly as it does in other mammalian species. In food-restricted female rats [15, 92] and ewes [93], and in fasted heifers [94] and male rhesus monkeys [95], a single ad libitum meal stimulates LH pulses within 2 hours. Such observations have been interpreted to imply that the physiological signals produced by a single large meal are sufficient to activate the hypothalamic GnRH neurons that control LH pulsatility [96].

We suspected that the restoration of LH pulsatility by refeeding might be considerably slower in energetically disrupted women than in other mammals, because the human brain requires so much more energy than does the brain of any other mammal. The brain competes against all

other tissues of the body for energy, and the adult human brain requires 20% of basal metabolic energy, compared to only 2% for most species and 8% for nonhuman primates [97]. Therefore, we suspected that a single meal might not provide enough energy to activate GnRH neurons in energetically disrupted women.

To stringently test this hypothesis, we assayed LH in blood samples drawn from women at 10 minute intervals for 48 hours during the mid-follicular phase, first during 24 hours on the fifth day of low energy availability treatments and then during 24 hours of aggressive refeeding. A combination of moderate dietary energy restriction (25 kcal/kgLBM/day) and moderate exercise energy expenditure (15 kcal/kgLBM/day) was administered to impose a low energy availability of 10 kcal/kgLBM/day. The aggressive refeeding regimen was comprised of 15 meals providing a total of 85 kcal/kgLBM/day. Combined with the same exercise treatment, the energy availability during the 24 hours of aggressive refeeding was 70 kcal/kgLBM/day.

Compared to measurements of LH pulsatility in 18 other women studied previously in our laboratory under balanced energy availability conditions and at the same phase of the menstrual cycle, low energy availability suppressed LH pulsatility unambiguously in five of the eight subjects treated in this experiment. Their LH pulse frequency was reduced 57% to 8.2 ± 1.5 pulses/24 hours, well below the 5th percentile of LH pulse frequencies in energy balanced women (14.6 pulses/24 hours), while their LH pulse amplitude was increased 94% to 3.1 ± 0.3 IU/L, well above the 95th percentile of LH pulse amplitudes in energy balanced women (2.5 IU/L).

Amongst these women, aggressive refeeding raised LH pulse frequency by only 2.4 ± 1.0 pulses/24 hours, still far below the 5th percentile of LH pulse frequency in energy balanced women. Meanwhile, the unambiguously elevated LH pulse amplitude was completely unaffected (Δ = 0.0 ± 0.4 IU/L) by aggressive refeeding. Results were similar when all eight subjects were included in the analysis. Aggressive refeeding pushed the group as a whole to, but not past, the 5th and 95th percentiles of LH pulse frequency and amplitude, respectively. Thus, as we had suspected, 24 hours of a refeeding protocol much more aggressive than the ad libitum refeeding protocols commonly employed in animal experiments had very little restorative effect on LH pulsatility in our energetically suppressed women.

Excalibur V

In an experimental protocol similar to that of Excalibur II, Excalibur V determined the dose-response effects of low energy availability on LH pulsatility in habitually sedentary, regularly menstruating young women [28]. To do this, we administered balanced and one of three low energy availabilities (45 and either 10, 20, or 30 kcal/kgLBM/day) to healthy, habitually sedentary, regularly menstruating women for 5 days. The design is illustrated in Fig. 11.3.

We found that LH pulsatility was disrupted within 5 days below a threshold of energy availability at ~30 kcal/kgLBM/day (Fig. 11.4). This was, in fact, the same actual energy availability that we had reported as 25 kcal/kgLBM/day in Excalibur II [80], because we had underestimated energy availability by 5 kcal/kgLBM/day in Excalibur II, as described in the discussion of Excalibur III above.

The disruption of LH pulsatility below 30 kcal/kgLBM/day in Excalibur V was consistent with many observational studies of amenorrheic runners, all of which indicated energy availabilities less than 30 kcal/kgLBM/day [98]. It was also consistent with the only prospective study of the refeeding of amenorrheic athletes, in which menstrual cycles had been restored in runners by increasing their energy availability from 25 to 31 kcal/kgLBM/day [99]. Energy availabilities below 30 kcal/kgLBM/day have also been reported in eumenorrheic athletes [98], 80% of whom display subclinical ovarian disorders in which the suppression of progesterone may also impair fertility [100].

In the same experiment, we also determined the dose-response effects of low energy availability on several metabolic substrates and hormones. Down to an energy availability of 30 kcal/kgLBM/day, the responses of insulin,

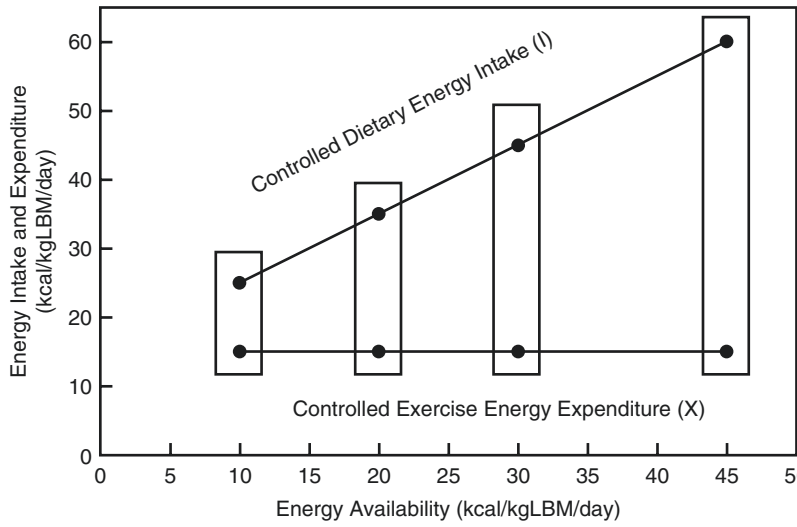

Fig. 11.3 Experimental design of Excalibur V. Women were assigned to contrasting energy availability treatments of 45 and 10, 45 and 20, and 45 and 30 kcal/kgLBM/day. All subjects performed a controlled exercise energy expenditure of 15 kcal/kgLBM/day in aerobic exercise at 70% VO_2 max under supervision, while their dietary energy intake was controlled to achieve the intended energy availabilities. (Reproduced with permission from [28], *Copyright 2003, The Endocrine Society*)

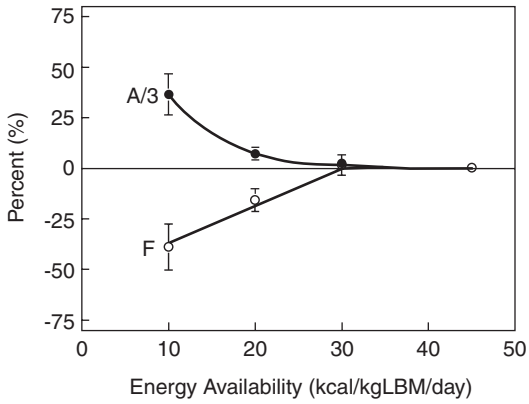

Fig. 11.4 Incremental effects of energy availability on LH pulse amplitude (A/3) and LH pulse frequency (F) in Excalibur V. Effects are expressed relative to values at 45 kcal/kgLBM/day. Effects on LH pulse amplitude have been divided by three for graphical symmetry. As energy availability declines from 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. (Reproduced with permission from [28], *Copyright 2003, The Endocrine Society*)

cortisol, insulin-like growth factor (IGF)-I/IGF-binding protein (IGFBP)-1, IGF-I/IGFBP-3, leptin, and T_3 maintained plasma glucose levels to within 3% of normal values. Below that threshold, however, plasma glucose levels fell despite further increases in the responses of the metabolic hormones, and effects on LH pulsatility appeared.

Excalibur V also revealed the dose-response effects of low energy availability on biochemical markers of bone turnover [101]. Urinary concentrations of N-telopeptide of type I collagen, a marker of the rate of whole body bone resorption, rose as estradiol concentrations declined, when energy availability was lowered to 10 kcal/kgLBM/d. By comparison, markers of bone formation declined at higher energy availabilities. Concentrations of serum carboxy-terminal propeptide of type I procollagen, a marker of bone type I collagen synthesis, and insulin declined linearly with energy availability. By contrast, concentrations of osteocalcin, a marker of bone mineralization, declined abruptly below 30 kcal/kgLBM/day together with IGF-I and T_3, which modulates the hepatic synthesis of IGF-I in response to GH stimulation. Such uncoupling of bone turnover, with increased resorption and reduced formation, can lead to irreversible reductions in bone mineral density [102].

Excalibur VI

The prevalence of amenorrhea has been reported to decline from 67% in marathon runners younger than 15 years of gynecological age to only 9% in those who were older [103]. Meanwhile, in the general population, the incidence of menstrual disorders declines during the decade after menarche as fertility increases [104]. Excalibur VI investigated whether these two observations might both be explained by a declining sensitivity of LH pulsatility to low energy availability as the energy cost of growth decreases [29]. Calcium balance, which is an index of growth, does not decline to zero until 14 years of gynecological age [105].

In Excalibur VI, contrasting balanced and low energy availabilities (45 and 10 kcal/kgFFM/day) were administered to healthy, habitually sedentary, regularly menstruating, older adolescent women (5–8 years of gynecological age, ~20 years of calendar age) and young adult women (14–18 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 [29].

This insensitivity of LH pulsatility to energy deficiency in adult women was subsequently confirmed by a corresponding insensitivity of ovarian function to energy deficiency [106]. In that experiment, the energy availability of women 25–40 years of age was reduced to ~25 kcal/kgFFM/day for 4 months by a combination of dietary restriction (~600 kcal/day) and exercise (~200 kcal/day). This subthreshold energy deficiency reduced the body fatness of these reproductively mature women from 32% to 27% but caused no more than a mild suppression of luteal function.

An adult reproductive system that is more robust against insults of energy deficiency may be explained by a greater availability of glucose to the brain in adults than in adolescents at the same energy availabilities. This might occur if peripheral tissues in full-grown adults do not compete as aggressively against the brain for available energy or carbohydrate. Alternatively, the sensitivity of sensors in the central nervous system to signals of energy deficiency may decline during adolescence. These possibilities remain to be investigated.

Other Efforts to Manipulate Energy Availability in Habitually Sedentary Women

A recent study by Lieberman et al. [107] investigated the effects of energy availability on menstrual function by reanalyzing data collected in an earlier experiment that had attempted to administer controlled negative energy balance (NEB) treatments of −15%, −30%, and −60% to separate groups of habitually sedentary regularly menstruating women for 3 months [108]. Thirty-five women with 5–15 years of gynecological age and ovulatory cycles as long as 35 days were studied, even though cycles of 36 days were to be classified as a clinical menstrual disturbance (oligomenorrhea) and the average within-person annual standard deviation of cycle length at the subjects' age is 4 days [85].

In practice, NEB turned out to be less negative and more widely dispersed than intended (mean ± 2SD, −8 ± 10%, −22 ± 21%, and −42 ± 9%). Moreover, metabolic hormone indicators of energy deficiency did not display dose-response effects of group differences in NEB. Assuming the underlying diet and exercise data were correct, Lieberman et al. calculated energy availability values in each menstrual cycle and found a continuum of energy availability treatments from 18 to 51 kcal/kgFFM/day.

Unfortunately, Lieberman et al. did not report the effects on metabolic hormones. They found no dose-response effects of energy availability on ovarian steroids. Altogether, they found that 36% of 105 menstrual cycles across the range of energy availability displayed menstrual disturbances and 85% of these were subclinical (luteal phase deficiency and anovulation). Collectively, only one menstrual cycle was missed.

Lieberman et al. concluded that their results "do not support that a threshold energy availability exists below which menstrual disturbances are induced," thereby appearing to confirm the

Female Athlete Triad as a continuum of interrelated disorders. However, given the 10–15% incidence of oligomenorrhea and the 15–65% incidence of subclinical menstrual disturbances in free-living women of the same gynecological age [109, 110], the observations of Lieberman et al. are better interpreted as what would be expected without any intervention. Moreover, without a crossover design and without dose-response effects of energy availability on any physiological indicator of energy deficiency, Lieberman et al. simply lack evidence that the disturbances they observed were caused by the treatments administered.

Reversal of Amenorrhea in Amenorrheic Athletes

Cialdella-Kam et al. [111] administered a carbohydrate-protein dietary supplement of 360 kcal/day to athletes with clinical menstrual disorders (7 amenorrheic and 1 oligomenorrheic). After 6 months, the eight athletes had resumed menses with seven of them resuming ovulation. However, it should be noted that the investigators calculated EA without subtracting non-exercise energy expenditure NEEE. Therefore, as they acknowledged in another paper [112], their pre and post EA values probably underestimated actual EA values by 1–2 kcal/kgFFM/day. Prior to this study, there had been pilot studies published of amenorrheic athletes who increased caloric intake for several months, and changes in menstrual status were observed [99, 113].

Pre and post EA values depended, of course, on the definition of exercise. When exercise was defined as activity when energy expenditure was greater than 4.0 METS, the dietary supplement increased energy availability from 37 to 45 kcal/kgFFM/day ($p = 0.10$). However, when exercise was defined more broadly to include all planned exercise plus bicycle commuting and all walking, energy availabilities were lower with the dietary supplement increasing them from 28 when amenorrheic to 39 kcal/kgFFM/day after restoration of menses ($p = 0.09$) [112].

More prospective research is needed to determine successful behavioral strategies that amenorrheic athletes with low energy availability can use to resume menstrual status. For example, research on appetite suppression by exercise and dietary restriction suggests that it may be important for athletes to consume planned amounts of energy at planned times, by discipline instead of appetite [114].

Conclusions About the Hypothetical Mechanisms of Functional Hypothalamic Amenorrhea in Female Athletes

We are unaware of any experiments that have determined the independent effect of body composition on the HPG axis. From the available experimental data, however, it would appear to be more likely that a lean body composition and disruption of the HPG axis are both effects of low energy availability than that a lean body composition disrupts the HPG axis. Our short-term experiments on women have demonstrated that exercise stress has no suppressive effect on LH pulsatility beyond the impact of the energy cost of the exercise on energy availability. These short-term 4–5-day experiments investigating the independent effects of exercise stress and low energy availability on LH pulsatility predicted and, as we expected, were later confirmed by long-term experiments investigating the independent effects of exercise stress and low energy availability on estrus and menstrual cycles. Prospective controlled experiments on both humans and animal models have demonstrated that the factor disrupting the HPG axis in physically active women is low energy availability. These experiments suggest that women may be able to prevent or to reverse menstrual disorders by dietary reform alone without moderating their exercise regimen. As long as dietary energy intake is managed to keep energy availability above 30 kcal/kgLBM/day, there may be no need to interfere with endurance, strength, and skill training. Finally, the susceptibility of women to the disruption of reproductive function by energy deficiency appears to be substantially greater in those younger than 15 years of gynecological age.

Causes of Low Energy Availability in Female Athletes

Effective treatment of low energy availability in athletes requires that the origin of the low energy availability be identified. Low energy availability behaviors appear to derive from four different origins [1, 114]. Some athletes intentionally reduce energy availability in a rational, but misguided, pursuit of the body size, body composition, and mix of metabolic fuel stores that are thought to optimize performance in their particular sport. Complex objectives may include reducing fat mass while increasing muscle mass and maximizing glycogen stores. For such athletes who reduce energy availability excessively, nutrition education and guidance regarding appropriate, individualized intermediate and ultimate goals, schedules, and methods may be sufficient to modify their diet and exercise behavior.

In other athletes, low energy availability originates in an eating disorder. Eating disorders are clinical mental illnesses that are often accompanied by other mental illnesses [115, 116]. Therefore, eating disorders require psychiatric treatment, often inpatient treatment, as well as nutritional counseling. Because the mortality of eating disorders is so high, sports organizations need to develop institutional methods for distinguishing undernourished athletes with eating disorders from those who do not have eating disorders. This distinction may not be obvious, since undernourished athletes who are only trying to optimize performance may practice many of the same disordered eating behaviors (e.g., skipping meals, vomiting, using laxatives, etc.) as athletes with eating disorders. Athletes with eating disorders are distinctive in their resistance to the efforts of coaches, trainers, nutritionists, and physicians to modify their behavior.

The third origin of low energy availability in athletes is the suppression of appetite by prolonged exercise. This effect is compounded by the appetite-suppressing effect of diets containing high percentages of carbohydrates, which are commonly recommended to athletes in endurance sports. Even though many studies on this subject have been published over the past 20 years [114, 117], appetite remains a largely neglected topic in the field of sports nutrition. Indeed, the word "appetite" appears only twice, in the recently revised joint position stand of the American Dietetic Association, the Dietitians of Canada, and the American College of Sports Medicine on nutrition and athletic performance [118].

Briefly, food deprivation increases hunger, but the same energy deficit produced by exercise energy expenditure does not [119]. The appetite-suppressing effect of prolonged exercise has been demonstrated in controlled experiments with protocols ranging from a few hours to 12 weeks [114]. The effect is mediated by the orexigenic hormone ghrelin, which induces us to begin eating, and by several anorexigenic hormones (including peptide YY, glucagon-like peptide 1, and pancreatic polypeptide) that induce us to stop eating. Exercise does not stimulate an increase in ghrelin concentrations but does stimulate increases in the concentrations of anorexigenic hormones (see associated Chaps. 12 and 30). As a result, "there is no strong biological imperative to match energy intake to activity-induced energy expenditure" [120].

Meanwhile, the appetite-suppressing effect of diets containing high percentages of carbohydrates has been demonstrated in experimental protocols ranging from a week [121] to a month [122, 123]. As the percentage of carbohydrates in the diet was reduced, ad libitum energy intake spontaneously increased. As a result, the actual amount of carbohydrate consumed was preserved even though the percentage of carbohydrates in the diet decreased from 67% to 55%. The mechanism of this effect has not yet been identified but may involve the greater bulk and fiber content of carbohydrate-rich foods.

Importantly, the large effects of these two factors are additive [121] so that together they can reduce energy availability below 30 kcal/kgFFM/day in endurance athletes. To avoid inadvertent low energy availability, therefore, athletes in endurance sports need to be trained to eat by discipline (i.e., planned amounts of selected foods at scheduled times) instead of appetite.

The fourth apparent origin of low energy availability among female athletes is that young

women under-eat for social reasons unrelated to sport. Around the world, about twice as many young women as young men at every decile of body mass index perceive themselves to be overweight, and the numbers actively trying to lose weight are even more disproportionate [124]. Alarmingly, the disproportion even *increases* as BMI declines, so that almost 9 times as many lean women as lean men are actively trying to lose weight! Indeed, more young female athletes report improvement of appearance than improvement of performance as a reason for dieting [125]. As a result, social issues unrelated to sport may need to be addressed to persuade female athletes to eat by discipline *beyond* their appetites.

Sources of Error in the Estimation and Control of EA

In publications of the Excalibur experiments, the portion of body composition apart from fat mass is termed LBM. It is better termed fat-free mass (FFM). Then, as currently understood, energy availability (EA) is quantified by measuring dietary energy intake (EI), exercise energy expenditure (EEE), and fat-free mass (FFM). EA is then calculated as:

$$EA = (EI - EEE) / FFM$$

A common source of error (by us in Excalibur I and II and by others) in studies of EA in athletes has derived from the misunderstanding of EEE as the total energy expenditure that would be measured by an ergometer during exercise. *This misunderstanding has led to underestimations of EA, misinterpretations of data, and unwarranted criticisms of the concept.*

As described in the discussion of Excalibur III above, EEE is defined as the *extra* energy expended *beyond* the energy that would have been expended if no exercise had been performed (see Fig. 11.5). Defining EEE in this way enables EA to be fairly compared between different groups of subjects who do and do not exercise and between repeated observations of the same subjects when they do and do not exercise. Because energy expenditure varies with routine activities during the day, to calculate EA consistently with the Excalibur experiments, non-exercise energy expenditure (NEEE) must be measured on another non-exercising day during the same waking hours when exercise is performed. Then EEE is calculated as the difference between total energy expenditure during exercise (TEEE) and NEEE on the other day:

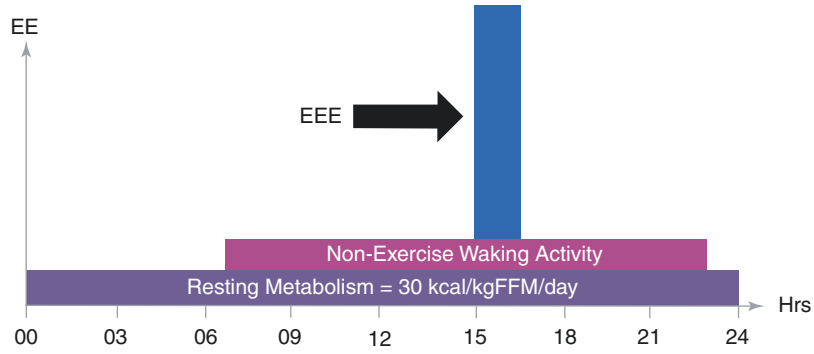

Fig. 11.5 Calculation of exercise energy expenditure (EEE). (A. Top) EEE is the amount of energy that a woman expends because she is an athlete and does not include the energy she expends in resting metabolism and other waking activities. (B. Middle) Ergometers measure total energy expenditure during exercise (TEEE), which overestimates EEE by ~2 kcal/kgFFM/d per hour of exercise. For high-intensity exercise of short duration, the resulting error in calculating energy availability as EA = (EI – TEEE)/FFM is negligibly small for clinical purposes. (C. Bottom) For low-intensity exercise of long duration, however, the error in EA = (EI – TEEE)/FFM is very large and will lead to unwarranted changes in diet and exercise behavior. (Adapted from [126])

$$EEE = TEEE - NEEE$$

In an example described in a previous review [126], the resting metabolism (RM) of an athlete in energy balance on a non-exercising day is assumed to be 2/3 of her EI. For EI = 2100 kcal/day (8.8 MJ/day), RM = 1400 kcal/day (5.8 MJ/day) or 58 kcal/hour (244 kJ/h). If she sleeps 8 hours, her routine activities in waking energy expenditure (WEE) would expend the rest of her EI. Ignoring for simplicity other sources of diurnal variation in energy expenditure, her average rate of WEE would be 700 kcal/16 hours = 44 kcal/h (182 kJ/h). If her fat-free mass (FFM) is 45 kg, then her rate of non-exercise energy expenditure (NEEE) during exercise would be:

$$NEEE = (RM + WEE)/FFM = (58 + 44)/45 = 2.3\,\text{kcal/kgFFM/h}\,(9.5\,\text{kJ/h})$$

If the athlete's total energy expenditure during a 40-minute run is TEEE = 500 kcal, then:

$$EEE = TEEE - NEEE = 500/45 - (2/3) * 2.3 = 11.1 - 1.5 = 9.6\,\text{kcal/kgFFM}$$

For such brief, high-intensity exercise, NEEE (1.5 kcal/kgFFM) is too small to cause an error in judgment about the adequacy of EA. However, if the same TEEE had been expended in 4 hours of gymnastics training, NEEE (9.2 kcal/kgFFM) would be too large to ignore:

$$EEE = TEEE - NEEE = 500/45 - 4 * 2.3 = 11.1 - 9.2 = 1.9\,\text{kcal/kgFFM}$$

If this gymnast were to restrict her dietary intake to EI = 1575 kcal/day, ignoring NEEE would lead to excessive concern about her EA and unwarranted demands for behavior modifications:

With NEEE:

$$EA = (EI - EEE)/FFM = 1575/45 - 1.9 = 33.1\,\text{kcal/kgFFM/day}\,(138\,\text{kJ/kgFFM/day})$$

Ignoring NEEE:

$$(EA = EI - TEEE)/FFM = 1575/45 - 9.6 = 25.4\,\text{kcal/kgFFM/day}\,(106\,\text{kJ/kgFFM/day})$$

Other sources of error in the calculation of EA derive from errors in the estimation of EI, EEE, and FFM. As pointed out in another review [126], a few simple calculations with realistic values quickly reveal that the greatest efforts should be made to record EI accurately. Consider an athlete with body mass = 60 kg, %Fat = 25%, EEE = 500 kcal/day, and EI = 2100 kcal/day (8.8 MJ/day). Her FFM is (1–0.25) × 60 = 45 kg and her EA is

$$EA = (EI - EEE)/FFM = (2100 - 500)/45 = 35.6\,\text{kcal/kgFFM/day}\,(149\,\text{kJ/kgFFM/day})$$

A 2% error rate in %Fat determinations is not uncommon with body composition analyzers. Subsequently, a 2% overestimate of %Fat (i.e., 27% in the above example) leads to an underestimate of FFM (43.8 kg) and a negligible error in EA:

$$EA = (2100 - 500)/43.8 = 36.5\,\text{kcal/kgFFM/day}\,(153\,\text{kJ/kgFFM/day})$$

A 10% error in EEE would correspond to a runner erring by half a mile in the length of a 5-mile run. A 10% underestimation of EEE leads to a similarly negligible error in EA:

$$EA = (2100 - 450)/45 = 36.7\,\text{kcal/kgFFM/day}\,(153\,\text{kJ/kgFFM/day})$$

Underestimations of EI as big as 20% have been suspected by some dietitians. A 20% underestimation of EI would lead to a large error in EA:

$$EA = (0.8 \times 2100 - 500)/45 = 26.2\,\text{kcal/kgFFM/day}\,(110\,\text{kJ/kgFFM/day})$$

Even a 10% underestimation of EI would lead to a substantial error in EA:

$$EA = (0.9 \times 2100 - 500)/45 = 30.9\,\text{kcal/kgFFM/day}\,(129\,\text{kJ/kgFFM/day})$$

A 10% error in EI (210 kcal) is similar to the energy content of 2–3 slices of bread. If EI is underestimated by 10–20%, then these substantial errors in EA will lead to misinterpretations of experimental data and mismanagement of athletes. Therefore, accurate estimations of EA depend most importantly on complete dietary records. In the Excalibur experiments, the accuracy of EI treatments was achieved by administering and supervising known meals. Difficult as that is for investigators and participants alike, quantifying EI in observational studies of free-living athletes is even more challenging.

Conclusion: Needed Research

More short-term experiments are needed to resolve the ambiguity about whether LH pulsatility depends on energy in general or on specific macronutrients in particular. Clinical trials are needed to verify that women can prevent or reverse functional hypothalamic amenorrhea by dietary reform alone without moderating the exercise regimen and to develop effective interventions that may be sport-specific. In addition, more animal experiments using the new modified activity stress paradigm ([90]) are needed to explore the physiological and neuroendocrine mechanisms of the Female Athlete Triad in more detail. Finally, more experiments like Excalibur III are needed to determine whether other stressors besides exercise have any suppressive effect on LH pulsatility beyond the impact of their energy cost on energy availability. Long-term experiments like Excalibur V are needed to look at EA threshold effects on other aspects of reproductive function besides LH pulsatility, but the expense and controls needed to properly conduct such studies make them challenging to conduct.

Conflict of Interest Anne Loucks is a founder and shareholder of AEIOU Scientific, LLC.

References

1. Nattiv A, Loucks AB, Manore MM, Sundgot-Borgen J, Warren MP. American College of Sports Medicine Position Stand. The Female Athlete Triad. Med Sci Sports Exerc. 2007;39(10):1867–82.
2. Otis CL, Drinkwater B, Johnson M, Loucks A, Wilmore J. American College of Sports Medicine position stand. The Female Athlete Triad. Med Sci Sports Exerc. 1997;29(5):i–ix.
3. De Souza MJ, Williams NI, Nattiv A, Joy E, Misra M, Loucks AB, et al. Misunderstanding the Female Athlete Triad: refuting the IOC consensus statement on Relative Energy Deficiency in Sport (RED-S). Br J Sports Med. 2014;48(20):1461–5.
4. Joy E, De Souza MJ, Nattiv A, Misra M, Williams NI, Mallinson RJ, et al. 2014 Female Athlete Triad Coalition consensus statement on treatment and return to play of the Female Athlete Triad. Curr Sports Med Rep. 2014;13(4):219–32.
5. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, et al. The IOC consensus statement: beyond the Female Athlete Triad–Relative Energy Deficiency in Sport (RED-S). Br J Sports Med. 2014;48(7):491–7.
6. Frisch RE, McArthur JW. Menstrual cycles: fatness as determinant of minimum weight for height necessary for their maintenance or onset. Science. 1974;185:949–51.
7. Frisch RE, Revelle R. Height and weight at menarche and a hypothesis of menarche. Arch Dis Child. 1971;46(249):695–701.
8. Schneider JE, Wade GN. Control of fertility by metabolic cues – reply. Am J Physiol Endocrinol Metab. 1997;273(1):E231–E2.
9. Crist DM, Hill JM. Diet and insulin like growth factor I in relation to body composition in women with exercise-induced hypothalamic amenorrhea. J Am Coll Nutr. 1990;9(3):200–4.
10. Bronson FH, Manning JM. The energetic regulation of ovulation: a realistic role for body fat. Biol Reprod. 1991;44(6):945–50.
11. Loucks AB, Horvath SM. Athletic amenorrhea: a review. Med Sci Sports Exerc. 1985;17(1):56–72.
12. Scott EC, Johnston FE. Critical fat, menarche, and the maintenance of menstrual cycles: a critical review. J Adolesc Health Care. 1982;2(4):249–60.
13. Sinning WE, Little KD. Body composition and menstrual function in athletes. Sports Med. 1987;4(1):34–45.
14. Loucks AB, Horvath SM, Freedson PS. Menstrual status and validation of body fat prediction in athletes. Hum Biol. 1984;56(2):383–92.

15. Bronson FH. 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. 1986;118(6):2483–7.
16. Di Carlo C, Palomba S, De Fazio M, Gianturco M, Armallino M, Nappi C. Hypogonadotropic hypogonadotropism in obese women after biliopancreatic diversion. Fertil Steril. 1999;72(5):905–9.
17. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425–32.
18. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 1996;334(5):292–5.
19. Tena-Sempere M. Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology. 2007;86(3):229–41.
20. Castellano JM, Bentsen AH, Mikkelsen JD, Tena-Sempere M. Kisspeptins: bridging energy homeostasis and reproduction. Brain Res. 2010;1364:129–38.
21. Chou SH, Chamberland JP, Liu X, Matarese G, Gao C, Stefanakis R, et al. Leptin is an effective treatment for hypothalamic amenorrhea. Proc Natl Acad Sci U S A. 2011;108(16):6585–90.
22. Ma Z, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M. Radioimmunoassay of leptin in human plasma. Clin Chem. 1996;42(6 Pt 1):942–6.
23. Laughlin GA, Yen SSC. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab. 1997;82(1):318–21.
24. Mantzoros C, Flier JS, Lesem MD, Brewerton TD, Jimerson DC. Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. J Clin Endocrinol Metab. 1997;82(6):1845–51.
25. Miller KK, Parulekar MS, Schoenfeld E, Anderson E, Hubbard J, Klibanski A, et al. Decreased leptin levels in normal weight women with hypothalamic amenorrhea: the effects of body composition and nutritional intake. J Clin Endocrinol Metab. 1998;83(7):2309–12.
26. Jimerson DC, Mantzoros C, Wolfe BE, Metzger ED. Decreased serum leptin in bulimia nervosa. J Clin Endocrinol Metab. 2000;85(12):4511–4.
27. Hilton LK, Loucks AB. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am J Physiol Endocrinol Metab. 2000;278(1):E43–9.
28. Loucks AB, Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab. 2003;88(1):297–311.
29. Loucks AB. The response of luteinizing hormone pulsatility to five days of low energy availability disappears by 14 years of gynecological age. J Clin Endocrinol Metab. 2006;91:3158–64.
30. Berga SL, Marcus MD, Loucks TL, Hlastala S, Ringham R, Krohn MA. Recovery of ovarian activity in women with functional hypothalamic amenorrhea who were treated with cognitive behavior therapy. Fertil Steril. 2003;80(4):976–81.
31. Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, et al. Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. Diabetes. 1996;45(11):1511–5.
32. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab. 1996;81:4162–5.
33. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Clin Endocrinol Metab. 1997;82:561–5.
34. Jenkins AB, Markovic TP, Fleury A, Campbell LV. Carbohydrate intake and short-term regulation of leptin in humans. Diabetologia. 1997;40(3):348–51.
35. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature. 1998;393(6686):684–8.
36. Warren MP. The effects of exercise on pubertal progression and reproductive function in girls. J Clin Endocrinol Metab. 1980;51:1150–7.
37. Winterer J, Cutler GB Jr, Loriaux DL. Caloric balance, brain to body ratio, and the timing of menarche. Med Hypotheses. 1984;15:87–91.
38. Wade GN, Schneider JE. Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev. 1992;16(2):235–72.
39. Bronson FH, Heideman PD. Seasonal regulation of reproduction in mammals. In: Knobil E, Neill J, editors. The physiology of reproduction, vol. 2. New York: Raven Press; 1994. p. 541–83.
40. Bronson FH, Manning J. Food consumption, prolonged exercise, and LH secretion in the peripubertal female rat. In: Pirke KM, Wuttle W, Schweiger U, editors. The menstrual cycle and its disorders. Berlin: Springer-Verlag; 1989. p. 42–9.
41. Wade GN, Schneider JE, Li HY. Control of fertility by metabolic cues. Am J Phys. 1996;270(1 Pt 1):E1–19.
42. Furman M, Wade GN. Animal models in the study of nutritional infertility. Curr Opin Endocrinol. 2007;14(6):475–81.
43. Laughlin GA, Yen SSC. Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. J Clin Endocrinol Metab. 1996;81(12):4301–9.
44. Christo K, Cord J, Mendes N, Miller KK, Goldstein MA, Klibanski A, et al. Acylated ghrelin and leptin in adolescent athletes with amenorrhea, eumenorrheic athletes and controls: a cross-sectional study. Clin Endocrinol. 2008;69(4):628–33.
45. Scheid JL, Williams NI, West SL, VanHeest SL, De Souza MJ. Elevated PYY is associated with energy deficiency and indices of subclinical disordered eating in exercising women with hypothalamic amenorrhea. Appetite. 2009;52(1):184–92.
46. Myerson M, Gutin B, Warren MP, May MT, Contento I, Lee M, et al. Resting metabolic rate and energy bal-

ance in amenorrheic and eumenorrheic runners. Med Sci Sports Exerc. 1991;23:15–22.
47. Loucks AB, Laughlin GA, Mortola JF, Girton L, Nelson JC, Yen SSC. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. J Clin Endocrinol Metab. 1992;75(2):514–8.
48. Loucks AB, Mortola JF, Girton L, Yen SS. Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. J Clin Endocrinol Metab. 1989;68(2):402–11.
49. Drinkwater BL, Nilson K, Chesnut CH 3rd, Bremner WJ, Shainholtz S, Southworth MB. Bone mineral content of amenorrheic and eumenorrheic athletes. N Engl J Med. 1984;311(5):277–81.
50. Kaiserauer S, Snyder AC, Sleeper M, Zierath J. Nutritional, physiological, and menstrual status of distance runners. Med Sci Sports Exerc. 1989;21(2):120–5.
51. Marcus R, Cann C, Madvig P, Minkoff J, Goddard M, Bayer M, et al. Menstrual function and bone mass in elite women distance runners. Endocrine and metabolic features. Ann Intern Med. 1985;102(2):158–63.
52. Nelson ME, Fisher EC, Catsos PD, Meredith CN, Turksoy RN, Evans WJ. Diet and bone status in amenorrheic runners. Am J Clin Nutr. 1986;43(6):910–6.
53. Edwards JE, Lindeman AK, Mikesky AE, Stager JM. Energy balance in highly trained female endurance runners. Med Sci Sports Exerc. 1993;25(12):1398-404.
54. Wilmore JH, Wambsgans KC, Brenner M, Broeder CE, Paijmans I, Volpe JA, et al. Is there energy conservation in amenorrheic compared with eumenorrheic distance runners? J Appl Physiol. 1992;72(1):15–22.
55. Mertz W, Tsui JC, Judd JT, Reiser S, Hallfrisch J, Morris ER, et al. What are people really eating? The relation between energy intake derived from estimated diet records and intake determined to maintain body weight. Am J Clin Nutr. 1991;54(2):291–5.
56. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med. 1995;332(10):621–8.
57. Rivier C, Rivest S. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol Reprod. 1991;45:523–32.
58. Selye H. The effect of adaptation to various damaging agents on the female sex organs in the rat. Endocrinology. 1939;25:615–24.
59. Asahina K, Kitahara F, Yamanaka M, Akiba T. Influence of excessive exercise on the structure and function of rat organs. Jpn J Physiol. 1959;9:322–6.
60. Axelson JF. Forced swimming alters vaginal estrous cycles, body composition, and steroid levels without disrupting lordosis behavior or fertility in rats. Physiol Behav. 1987;41(5):471–9.
61. Chatterton RT Jr, Hartman AL, Lynn DE, Hickson RC. Exercise-induced ovarian dysfunction in the rat. Proc Soc Exp Biol Med. 1990;193(3):220–4.
62. Manning JM, Bronson FH. Effects of prolonged exercise on puberty and luteinizing hormone secretion in female rats. Am J Phys. 1989;257(6 Pt 2):R1359–64.
63. Manning JM, Bronson FH. Suppression of puberty in rats by exercise: effects on hormone levels and reversal with GnRH infusion. Am J Phys. 1991;260(4 Pt 2):R717–23.
64. De Souza MJ, Maguire MS, Maresh CM, Kraemer WJ, Rubin KR, Loucks AB. Adrenal activation and the prolactin response to exercise in eumenorrheic and amenorrheic runners. J Appl Physiol. 1991;70(6):2378–87.
65. De Souza MJ, Luciano AA, Arce JC, Demers LM, Loucks AB. Clinical tests explain blunted cortisol responsiveness but not mild hypercortisolism in amenorrheic runners. J Appl Physiol. 1994;76(3):1302–9.
66. Ding J-H, Scheckter CB, Drinkwater BL, Soules MR, Bremner WJ. High serum cortisol levels in exercise-associated amenorrhea. Ann Intern Med. 1988;108:530–4.
67. Suh BY, Liu JH, Berga SL, Quigley ME, Laughlin GA, Yen SS. Hypercortisolism in patients with functional hypothalamic-amenorrhea. J Clin Endocrinol Metab. 1988;66(4):733–9.
68. Gold PW, Gwirtsman H, Averinos PC, Nieman LK, Gallucci WT, Kaye W, et al. Abnormal hypothalamic-pituitary-adrenal function in anorexia nervosa: pathophysiologic mechanisms in underweight and weight-corrected patients. N Engl J Med. 1986;314(21):1335–42.
69. Kuo T, Harris CA, Wang JC. Metabolic functions of glucocorticoid receptor in skeletal muscle. Mol Cell Endocrinol. 2013;380(1–2):79–88.
70. Slentz CA, Davis JM, Settles DL, Pate RR, Settles SJ. Glucose feedings and exercise in rats: glycogen use, hormone responses, and performance. J Appl Physiol. 1990;69:989–94.
71. Tabata I, Ogita F, Miyachi M, Shibayama H. Effect of low blood glucose on plasm CRF, ACTH, and cortisol during prolonged physical exercise. J Appl Physiol. 1991;71:1807–12.
72. Bonen A. Recreational exercise does not impair menstrual cycles: a prospective study. Int J Sports Med. 1992;13(2):110–20.
73. Boyden TW, Pamenter RW, Stanforth P, Rotkis T, Wilmore JH. Sex steroids and endurance running in women. Fertil Steril. 1983;39(5):629–32.
74. Bullen BA, Skrinar GS, Beitins IZ, Carr DB, Reppert SM, Dotson CO, et al. Endurance training effects on plasma hormonal responsiveness and sex hormone excretion. J Appl Physiol. 1984;56(6):1453–63.
75. Rogol AD, Weltman JY, Evans WS, Veldhuis JD, Weltman AL. Long-term endurance training alters the hypothalamic-pituitary axes for gonadotropins and growth hormone. Endocrinol Metab Clin North Am. 1992;21(4):817–32.
76. Loucks AB, Cameron JL, De Souza MJ. Subject assignment may have biased exercise results [letter; comment]. J Appl Physiol. 1993;74(4):2045–7.
77. Bullen BA, Skrinar GS, Beitins IZ, von Mering G, Turnbull BA, McArthur JW. Induction of menstrual disorders by strenuous exercise in untrained women. N Engl J Med. 1985;312(21):1349–53.

78. Williams NI, Young JC, McArthur JW, Bullen B, Skrinar GS, Turnbull B. Strenuous exercise with caloric restriction: effect on luteinizing hormone secretion. Med Sci Sports Exerc. 1995;27(10):1390–8.
79. Loucks AB, Callister R. Induction and prevention of low-T_3 syndrome in exercising women. Am J Phys. 1993;264(5 Pt 2):R924–30.
80. Loucks AB, Heath EM. Induction of low-T_3 syndrome in exercising women occurs at a threshold of energy availability. Am J Phys. 1994;266(3 Pt 2): R817–23.
81. Loucks AB, Heath EM. 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. 1994;78(4):910–5.
82. Loucks AB, Verdun M, Heath EM. Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. J Appl Physiol. 1998;84(1): 37–46.
83. Loucks AB, Verdun M. Slow restoration of LH pulsatility by refeeding in energetically disrupted women. Am J Phys. 1998;275(4 Pt 2):R1218–26.
84. Loucks A. Is stress measured in joules? Mil Psychol. 2009;21(S1):S101–S7.
85. Treloar AE, Boynton RE, Behn BG, Brown BW. Variation of the human menstrual cycle through reproductive life. Int J Fertil. 1967;12(1 Pt 2):77–126.
86. Olson BR, Cartledge T, Sebring N, Defensor R, Nieman L. Short-term fasting affects luteinizing hormone secretory dynamics but not reproductive function in normal-weight sedentary women. J Clin Endocrinol Metab. 1995;80(4):1187–93.
87. Alvero R, Kimzey L, Sebring N, Reynolds J, Loughran M, Nieman L, et al. Effects of fasting on neuroendocrine function and follicle development in lean women. J Clin Endocrinol Metab. 1998;83(1): 76–80.
88. Williams NI, Caston-Balderrama AL, Helmreich DL, Parfitt DB, Nosbisch C, Cameron JL. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: abrupt transition to exercise-induced amenorrhea. Endocrinology. 2001;142(6):2381–9.
89. Williams NI, Helmreich DL, Parfitt DB, Caston-Balderrama AL, Cameron JL. Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. J Clin Endocrinol Metab. 2001;86(11):5184–93.
90. DiMarco NM, Dart L, Sanborn C. Modified activity-stress paradigm in an animal model of the Female Athlete Triad. J Appl Physiol. 2007;103:1469–78.
91. Bursztein S, Elwyn DH, Askanazi J, Kinney JM. Fuel utilization in normal, starving, and pathological states. Energy metabolism, indirect calorimetry, and nutrition. Baltimore: Williams & Wilkins; 1989. p. 146.
92. Bronson FH, Heideman PD. Short-term hormonal responses to food intake in peripubertal female rats. Am J Phys. 1990;259(1 Pt 2):R25–31.
93. Foster DL, Ebling FJ, Micka AF, Vannerson LA, Bucholtz DC, Wood RI, et al. Metabolic interfaces between growth and reproduction. I. Nutritional modulation of gonadotropin, prolactin, and growth hormone secretion in the growth-limited female lamb. Endocrinology. 1989;125(1):342–50.
94. McCann JP, Hansel W. Relationships between insulin and glucose metabolism and pituitary-ovarian functions in fasted heifers. Biol Reprod. 1986;34(4):630–41.
95. Parfitt DB, Church KR, Cameron JL. Restoration of pulsatile luteinizing hormone secretion after fasting in rhesus monkeys (Macaca mulatta): dependence on size of the refeed meal. Endocrinology. 1991;129(2):749–56.
96. Schreihofer DA, Renda F, Cameron JL. Feeding-induced stimulation of luteinizing hormone secretion in male rhesus monkeys is not dependent on a rise in blood glucose concentration. Endocrinology. 1996;137(9):3770–6.
97. Flatt JP. Energetics of intermediary metabolism. In: Kinney JM, editor. Assessment of energy metabolism in health and disease. Columbus: Ross Laboratories; 1980. p. 77–87.
98. Loucks AB. Low energy availability in the marathon and other endurance sports. Sports Med. 2007;37(4–5):348–52.
99. Kopp-Woodroffe SA, Manore MM, Dueck CA, Skinner JS, Matt KS. Energy and nutrient status of amenorrheic athletes participating in a diet and exercise training intervention program. Int J Sport Nutr. 1999;9(1):70–88.
100. De Souza MJ, Miller BE, Loucks AB, Luciano AA, Pescatello LS, Campbell CG, et al. 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. 1998;83(12):4220–32.
101. Ihle R, Loucks AB. Dose-response relationships between energy availability and bone turnover in young exercising women. J Bone Mineral Res. 2004;19(8):1231–40.
102. Compston JE. Sex steroids and bone. Physiol Rev. 2001;81(1):419–47.
103. Baker ER, Mathur RS, Kirk RF, Williamson HO. Female runners and secondary amenorrhea: correlation with age, parity, mileage, and plasma hormonal and sex-hormone-binding globulin concentrations. Fertil Steril. 1981;36(2):183–7.
104. Ellison PT. Advances in human reproductive ecology. Annu Rev Anthropol. 1994;23:255–75.
105. Weaver CM, Martin BR, Plawecki KL, Peacock M, Wood OB, Smith DL, et al. Differences in calcium metabolism between adolescent and adult females. Am J Clin Nutr. 1995;61(3):577–81.
106. Williams NI, Reed JL, Leidy HJ, Legro RS, De Souza MJ. Estrogen and progesterone exposure is reduced in response to energy deficiency in women aged 25–40 years. Hum Reprod. 2010;25(9):2328–39.

107. Lieberman JL, DS MJ, Wagstaff DA, Williams NI. Menstrual disruption with exercise is not linked to an energy availability threshold. Med Sci Sports Exerc. 2018;50(3):551–61.
108. Williams NI, Leidy HJ, Hill BR, Lieberman JL, Legro RS, De Souza MJ. Magnitude of daily energy deficit predicts frequency but not severity of menstrual disturbances associated with exercise and caloric restriction. Am J Physiol Endocrinol Metab. 2015;308(1):E29–39.
109. Redman LM, Loucks AB. Menstrual disorders in athletes. Sports Med. 2005;35(9):747–55.
110. Vollman RF. The menstrual cycle. Major Probl Obstet Gynecol. 1977;7:1–193.
111. Cialdella-Kam L, Guebels CP, Maddalozzo GF, Manore MM. Dietary intervention restored menses in female athletes with exercise-associated menstrual dysfunction with limited impact on bone and muscle health. Nutrients. 2014;6(8):3018–39.
112. Guebels CP, Kam LC, Maddalozzo GF, Manore MM. Active women before/after an intervention designed to restore menstrual function: resting metabolic rate and comparison of four methods to quantify energy expenditure and energy availability. Int J Sport Nutr Exerc Metab. 2014;24(1):37–46.
113. Dueck CA, Matt KS, Manore MM, Skinner JS. Treatment of athletic amenorrhea with a diet and training intervention program. Int J Sport Nutr. 1996;6(1):24–40.
114. Loucks AB, Kiens B, Wright HH. Energy availability in athletes. J Sport Sci. 2011;S1:S7–S15.
115. Braun DL, Sunday SR, Halmi KA. Psychiatric comorbidity in patients with eating disorders. Psychol Med. 1994;24(4):859–67.
116. Kaye WH, Bulik CM, Thornton L, Barbarich N, Masters K. Comorbidity of anxiety disorders with anorexia and bulimia nervosa. Am J Psychiat. 2004;161(12):2215–21.
117. Loucks AB. Energy balance and body composition in sports and exercise. J Sport Sci. 2004;22:1–14.
118. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine joint position statement. Nutrition and athletic performance. Med Sci Sports Exerc. 2016;48(3):543–68.
119. Hubert P, King NA, Blundell JE. Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. Appetite. 1998;31(1):9–19.
120. Blundell JE, King NA. Physical activity and regulation of food intake: current evidence. Med Sci Sports Exerc. 1999;31(11 Suppl):S573–83.
121. Stubbs RJ, Hughes DA, Johnstone AM, Whybrow S, Horgan GW, King N, et al. Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. Am J Phys. 2004;286(2):R350–8.
122. Horvath PJ, Eagen CK, Ryer-Calvin SD, Pendergast DR. The effects of varying dietary fat on the nutrient intake in male and female runners. J Am Coll Nutr. 2000;19(1):42–51.
123. Horvath PJ, Eagen CK, Fisher NM, Leddy JJ, Pendergast DR. The effects of varying dietary fat on performance and metabolism in trained male and female runners. J Am Coll Nutr. 2000;19(1):52–60.
124. Wardle J, Haase AM, Steptoe A. Body image and weight control in young adults: international comparisons in university students from 22 countries. Int J Obesity. 2006;30(4):644–51.
125. Martinsen M, Bratland-Sanda S, Eriksson AK, Sundgot-Borgen J. Dieting to win or to be thin? A study of dieting and disordered eating among adolescent elite athletes and non-athlete controls. Br J Sport Med. 2010;44(1):70–6.
126. Loucks AB. The Female Athlete Triad: a metabolic phenomenon. Pensar En Movimiento. 2014;12(1):1–23.