Physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism in exercising women

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Amenorrhea associated with reduced caloric intake and strenuous exercise leads to hypoestrogenism and is associated with clinical manifestations that include disordered eating, stress fractures, osteoporosis, and, as recently reported, a potential increase in the risk of premature cardiovascular disease. Disordered eating, menstrual irregularities and bone loss comprise the clinical condition known as the ‘female athlete triad’. The aetiology of the triad is linked to a high prevalence of disordered eating and cognitive restraint. This results in inadequate caloric intake for high exercise energy expenditures that leads to energy deficiency. This in turn stimulates compensatory mechanisms, such as weight loss or energy conservation, subsequently causing a central suppression of reproductive function and concomitant hypoestrogenism. Because the aetiology of menstrual disturbances and the female athlete triad is causally linked to energy deficiency, there is no justification for fears that exercise itself is unhealthy for women. However, improved detection, monitoring, and treatment of all components of the triad in exercising women should be emphasized. This paper critically reviews the physiological aspects and clinical sequelae of energy deficiency and hypoestrogenism associated with the female athlete triad in exercising women.

Key words: amenorrhea/bone mineral density/female athlete triad/hypoestrogenism/luteal phase defects

Introduction

Amenorrhea associated with reduced caloric intake and strenuous exercise leads to hypoestrogenism and is associated with clinical manifestations that include disordered eating, stress fractures, osteoporosis, and, as recently reported, a potential increase in the risk of premature cardiovascular disease (Drinkwater et al., 1984, 1986, 1990; Otis et al., 1997; Zeni-Hoch et al., 2003). Specifically, studies of bone mineral density (BMD) in amenorrhoeic athletes (AA) show values equivalent to post-menopausal women, thereby increasing fracture risk (Drinkwater et al., 1984, 1986, 1990), and accounting for the high rate of stress fractures observed (Carbon et al., 1990; Otis et al., 1997; Korapelainen et al., 2001). Resumption of menses provides only limited BMD recovery (Drinkwater et al., 1990; Jonnavithula et al., 1993; Keen and Drinkwater, 1997). Recent reports of early signs of cardiovascular disease, i.e. endothelial dysfunction (Zeni-Hoch et al., 2003), combined with prior findings of unfavourable changes in lipid profiles (Lamon-Fava et al., 1989; Friday et al., 1993) in AA extend the clinical sequelae of hypoestrogenism in young women to include detrimental effects on cardiovascular function that could have health consequences later in life. The above-mentioned negative physiological effects on the skeletal and cardiovascular systems are presumably a direct result of the hypoestrogenism that accompanies the prolonged suppression of the reproductive axis. The aetiology of athletic amenorrhea is linked to a high prevalence of disordered eating and cognitive restraint (Sundgot-Borgen, 1994; Beals and Manore, 2001; McLean and Barr, 2003). Disordered eating and cognitive restraint can result in inadequate caloric intake for high exercise energy expenditures that, when combined, result in an energy deficit that stimulates compensatory mechanisms, such as weight loss or energy conservation, subsequently causing a central suppression of reproductive function (Sundgot-Borgen, 1994; Beals and Manore, 2001; Williams et al., 2001a,b; McLean and Barr, 2003).

The female athlete triad (Otis et al., 1997) is a syndrome of interrelated conditions that includes disordered eating, amenorrhoea and osteoporosis. The triad presumably develops due to internal and external pressures placed on athletes to maintain a low body weight (Sundgot-Borgen, 1994; Otis et al., 1997; Beals and Manore, 2000). The increasing presentation of the individual triad disorders is of growing concern, resulting from the increased numbers of females participating in competitive sports, especially at a younger age (Otis et al., 1997; Khan et al., 2002; Cobb et al., 2003). Furthermore, studies to date have limited their examination of the triad to athletic populations, failing to extend the scope of evaluations to recreationally physically active women. To date, the only clinical recommendations for prevention and treatment of amenorrhoea are that athletes...
increase their caloric intake and reduce their training (American Academy of Pediatrics, 2000). No specific dietary guidelines are available, and the rationale for reducing training may be an overly conservative approach, since exercise per se does not appear to play a causal role in athletic amenorrhoea (Loucks, 2003; Williams, 2003).

Prevalence of the female athlete triad

Importance of individual components of the triad

The female athlete triad has been scrutinized due to the lack of data available on the prevalence of the simultaneous occurrence of all three components of the triad, as defined in the original position stand of 1997 (DiPietro and Stachenfeld, 1997; Otis et al., 1997). A more informed understanding of the available data concerning the inter-relationships of the components of the triad does not require all three components to be present for significant health consequences to appear. Alone or in combination, each component of the triad warrants attention, as defined in the position stand. Reasons for the lack of data on the prevalence of the triad are multi-faceted. A useful framework for discussing the barriers for establishing prevalence is to examine this issue from multiple perspectives. First, issues related to detection of the triad and appropriate definitions of the components of the triad must be understood. Secondly, barriers facing frontline sports medicine providers, and the athletes themselves, should be addressed.

Defining and detecting the components of the triad

A more informed understanding acknowledges that each component exists on a continuum of severity, and that to apply the strict definitions for disordered eating, amenorrhoea and osteoporosis as defined in the original position stand is to underestimate the number of individuals who may benefit from detection and treatment of one or more components, to include sub-clinical presentations. On the other hand, detection of abnormalities necessitates standardized definitions, and our understanding of the most appropriate definitions to use for the components of the triad is still evolving. For example, some have argued that a more appropriate definition for bone loss associated with the triad might be the World Health Organization (WHO) criterion for osteopenia (Khan et al., 2002), although the International Society for Clinical Densitometry advises against the use of the WHO criteria for pre-menopausal women (Khan et al., 2002; Khan and Syed, 2004). Similarly, there is neither an accepted definition nor criteria for the diagnosis of ‘subclinical disordered eating’ (Beals and Manore, 2000), and criteria for defining subtle menstrual disturbances such as luteal phase defects (LPD) are varied (De Souza, 2003). Ongoing research that identifies an exact threshold at which point subclinical conditions produce negative health outcomes, or reliably predict risk of future negative health outcomes, is necessary to further clarify the definitions of the components of the triad and thereby improve prevention, detection and treatment.

With the relatively recent recognition of the triad in 1997, large scale studies are just beginning to emerge (Lauder et al., 1999; Cobb et al., 2003). The triad consists of three conditions that are most often recognized outside a clinical setting, i.e. in the training room, in the gym, or on the sidelines. Careful documentation of endocrine status to rule out other causes of amenorrhoea requires measurement of hormones, yet the acquisition of blood samples from athletes may be difficult. Other methods such as salivary and blood spot hormone testing are emerging and may represent useful alternatives for screening and detection of menstrual disturbances and energy deficits (Shirtcliff et al., 2001). Similarly, the measurement of BMD can be done with portable units, but these lack precision and accuracy (Chilibeck et al., 1994). Traditional BMD testing using dual-energy X-ray absorptiometry (DXA) is expensive and units dedicated for research are scarce. Self-report of disordered eating practices using established questionnaires that ask direct questions, such as the Eating Disorder Inventory (EDI) or Eating Attitude Test (EAT), have been shown to result in under-reporting and lying (McNulty et al., 2001), and consequently, surveys and interviews that utilize more indirect questioning methods are being developed (McNulty et al., 2001). Validation of these instruments in athletic populations must be completed before successful implementation for diagnosis of the triad occurs. Lastly, the gatekeeper to the athletes is often the coach. Researchers who try to gain access to athletic teams must often convince coaches that their research provides an outcome valuable to the coach, i.e. improvement in her/his team’s performance, before the researcher can proceed with the project.

Recognition of the triad by frontline sports medicine providers is difficult because education around the signs and symptoms of the triad is lacking, and because many universities and even more high schools have limited resources to support sports medicine professionals in their athletic programmes. The National Athletic Training Association (NATA) recently revised its guidelines for appropriate medical coverage of intercollegiate athletics after a 2 year epidemiological study was performed that documented higher injury rates in many sports (NATA, 2003). The guidelines called for increased numbers of trainers for prevention and treatment of injuries, but many universities fall short of the recommended ratio of sports medicine personnel to athletes required to treat routine injuries, let alone a complex syndrome like the triad (NATA, 2003). Unlike the triad, other clinical conditions such as breast cancer can be evaluated in large epidemiological studies with the help of established cancer registries or a clinical infrastructure that includes diagnosis tied to insurance reimbursement and an established standard of care.

From the perspective of the athlete, several reasons exist for either the denial or lack of recognition of the triad. First, as with coaches and other frontline personnel, knowledge regarding the signs and symptoms and inter-relatedness of the triad is lacking (Johnson et al., 1999). Many athletes hide pathogenic weight control behaviours or knowledge of their amenorrhoea due to fear of reprisal from coaches or medical personnel (Sanborn et al., 1987). Lastly, if a low body weight is desired in order to improve performance and/or please a coach or parent (Sundgot-Borgen, 1994), actions to address the adverse health consequences of the triad may be secondary to this goal.

To date, only two studies have simultaneously evaluated all aspects of the triad as defined in the original position stand; one study was in runners, i.e. athletes (Cobb et al., 2003), and the other in military women (Lauder et al., 1999). Most studies have evaluated the combination of two aspects of the triad, i.e. menstrual irregularities and bone health (Drinkwater et al., 1984, 1986, 1990; Lloyd et al., 1988; Marcus et al., 1985; Carbon et al.,
Sequelaes of energy deficiency and hypoestrogenism

Figure 1. The proposed expansion of the female athlete triad beyond the clinical sequelae of eating disorders, amenorrhea and osteoporosis to include increased cardiovascular risk other subclinical sequelae and incorporating recreationally physically active women among the affected.
of hypoestrogenism, in an expanded definition of the triad to include subclinical conditions, illustrated in Figure 1.

Amenorrhea

Ovarian function, and therefore female fertility, has been shown to vary naturally in humans according to ecological factors such as food availability, energy balance and energy expenditure that may change in response to seasonal or other environmental factors (Ellison, 1990; Ellison et al., 1993; Jasienska and Ellison, 1993). This variability in ovarian function, ranging from ovulatory menstrual cycles to amenorrhea, has been described in non-westernized subsistence communities in women who are impacted by seasonal food availability (Ellison, 1990; Ellison et al., 1993; Jasienska and Ellison, 1993). Menstrual cycle disturbances in athletes and active women in response to voluntary exercise and self moderation of food intake, have also been described as existing along a continuum of reproductive disturbances, ranging from subtle perturbations such as LPD and anovulatory cycles to the most severe presentation, amenorrhea (Ellison, 1990; De Souza, 2003) (Figure 2).

Amenorrhea is associated with the most extreme deficiency in estrogen (E2), while less severe menstrual perturbations have less severe deficits in E2. Amenorrhea is also associated with the most severe clinical sequelae (De Souza, 2003). In athletes, the amenorrhea is hypothalamic in origin with low and chronically suppressed levels of circulating gonadotrophins and ovarian steroids, and unaltered responsiveness of the pituitary gland and ovaries (Veldhuis et al., 1985). The definition of amenorrhea in the literature has varied (Loucks and Horvath, 1985), but should be conservatively defined as no menses for a minimum of 3 months. Reports of the prevalence ofamenorrhea in athletes range from 1 to 66% (Feicht et al., 1978; Dale et al., 1979; Schwartz et al., 1981; Sanborn et al., 1982; Carlberg et al., 1983; Loucks and Horvath, 1985), and grossly exceed estimates of this condition in sedentary women (2–5%) (Drew, 1961; Peterson et al., 1973). The highest prevalence of athletic amenorrhea is found in sports that emphasize a low body weight such as figure skating, ballet, long-distance running and gymnastics, but studies have documented menstrual abnormalities in a wide variety of sports (Feicht et al., 1978; Dale et al., 1979; Schwartz et al., 1981; Sanborn et al., 1982; Loucks and Horvath, 1985).

Delayed menarche

Delayed menarche, or primary amenorrhea, defined as failure to achieve menarche by age 16 years (Loucks and Horvath, 1985), has also been repeatedly reported in athletes participating in many sports, but particularly in ballet and gymnastics (Frisch et al., 1980; Warren, 1980; Peltenberg et al., 1984; Malina, 1994; Warren et al., 2003). The later ages at menarche in adolescent athletes are often attributed to regular exercise training for the respective sports without considering other factors known to influence delays in pubertal maturation (Malina, 1994). Malina (1994) states that in adequately nourished adolescents, the timing of menarche is very dependent on hereditary factor, but menarche is also influenced by a number of social or bio-cultural variables, including the self-selective nature of participation in some sports, such as gymnastics and ballet, where selection occurs for specific factors associated with later maturation.

Oligomenorrhea

Oligomenorrhea is defined by irregular and inconsistent menstrual cycles lasting from 36 to 90 days in length (Loucks and Horvath, 1985), and is a menstrual presentation that is difficult to study due to the nature of its inconsistent characteristics. As such, no definitive data exist on the prevalence of this menstrual abnormality in athletes, except to note that cycles of irregular length are often reported in female athletes (Loucks and Horvath, 1985) and the presence of oligomenorrhea has frequently been grouped together with amenorrhea in a number of studies (Tomten et al., 1998; Gremion et al., 2001; Csermely et al., 2002; Cobb et al., 2003). When daily measurement of hormones has not been feasible or affordable, investigators have also used definitions that have included four or fewer menstrual cycles per year to define oligomenorrhea (Cobb et al., 2003). The ovarian profile of an oligomenorrheic athlete displays erratic, unpredictable and presumably low E2 production as follicles struggle to achieve dominance. Oligomenorrheic cycles may be ovulatory or anovulatory, as the definitive event is the sloughing of the endometrial lining which can occur in response to increasing E2 levels that are independent of ovulation. Few examples of well-characterized oligomenorrheic cycles exist in the literature. Figure 3 illustrates actual patterns of urinary metabolites across several menstrual cycles in a previously sedentary eumenorrheic subject who underwent an intervention of aerobic exercise and a reduction in dietary intake and developed an oligomenorrheic cycle (N.I.Williams et al., unpublished data). It is important to note the relative degree of hypoestrogenism apparent in this oligomenorrheic cycle.

Anovulation

Anovulation is defined as the absence of an ovulatory event when LH and FSH secretion are low in association with reduced E2 levels and the absence of luteinization (Hamilton-Fairly and Taylor, 2003). Cycle length can vary in anovulatory cycles and therefore anovulation can be associated with oligomenorrhea. Although anovulation is characterized by low E2 and progesterone levels throughout the cycle, debate in the clinical forum continues regarding the specific criterion for confirming...
anovulation (Malcolm and Cumming, 2003). De Souza et al. (1998) have reported a 16% prevalence of anovulatory cycles, i.e. where menses occurs at regular (26–32 days) intervals but without an ovulatory event, in women who exercise at recreational levels, i.e. running, 12–15 miles a week. McConnell et al. (2002) have reported that ovulation could not be detected in 32% of Division 1 athletes from a wide variety of sports who reported regular menstrual bleeding (self report of cycles 26–32 days) (McConnell et al., 2002). In all cases, a higher level of E$_2$ is present in anovulatory cycles compared to amenorrhoeic cycles such that characterization of the cycle with daily ovarian steroid assessments is likely to show more E$_2$ production in a 30 day period than an equivalent period in an amenorrhoeic athlete (AA).

**Luteal phase defects**

LPD have been reported in women engaged in strenuous as well as recreational exercise (Ellison and Lager, 1986; Broocks et al., 1990; Beitins et al., 1991; Winters et al., 1996; De Souza et al., 1998; De Souza, 2003). In women with LPD, the ovarian system functions at a level adequate for ovulation, but inadequate to support implantation, since the latter is dependent on adequate progestational luteinization (Jones, 1976; Balasch and Vanrell, 1987). The presentation of LPD that occurs in athletes and physically active women is associated with reduced progesterone production during the luteal phase, and is also referred to as luteal phase inadequacy or insufficiency to describe the poor quality of the endometrium secondary to low progesterone levels. The low progesterone levels that occur are either low in volume or low in duration of output, since they occur in the face of normal menstrual cycle lengths of 26–32 days (Jones, 1976; Balasch and Vanrell, 1987). Women with exercise-associated LPD continue to ovulate although most do so much later than the typical mid-cycle day 12–14 in ‘normal’ ovulatory cycles. Ovulation can occur as late as day 20 or later (De Souza, 2003), reflecting a shortening of the luteal phase. The latter refers to luteal phases of ≤10 days that can present with or without progesterone inadequacy (Sherman and Korenman, 1974; Strott et al., 1970; Jones, 1976; De Souza, 2003). Clinically, LPD-associated progesterone inadequacy causes asynchronous follicular growth in the subsequent menstrual cycle, compromised oocyte maturation and differentiated (out of phase) function of the endometrium. All of the latter factors are associated with low rates of cycle fecundity and high rates of embryonic loss, i.e. infertility and spontaneous abortion (Jones, 1976; Balasch and Vanrell, 1987). In addition to changes in luteal length and progesterone concentrations, follicular phase length is prolonged in association with decreased output of daily urinary measures of E$_2$ apparent on days 2–12 of the follicular phase and during the luteal phase in exercising women with LPD (Winters et al., 1996; De Souza et al., 1998).

![Figure 3](image_url)

**Figure 3.** An actual example of an oligomenorrhoeic cycle from a subject in a prospective study examining the effects of an exercise and diet intervention on the menstrual cycle (unpublished data from N.I. Williams). The subject began the study with normal length, ovulatory cycles, then exercised five times per week for three menstrual cycles and consumed a hypocaloric diet (~30% fewer calories). During the last cycle of the intervention, this subject exhibits an oligomenorrhoeic cycle, denoted by the extended length compared to her earlier cycles. Ovulation could not be confirmed. PGD = pregnanediol 3-glucuronide; E1G = estrone glucuronide.

Sequelae of energy deficiency and hypoestrogenism
The prevalence of LPD in non-active, i.e. sedentary, women is controversial, but estimates vary from 2 to 5%, and from 3 to 20% in women with infertility (McNeely and Soules, 1988; De Souza et al., 1998). LPD occurs in athletes at a much greater prevalence than in non-active women, representing the most common menstrual cycle abnormality associated with exercise (McNeely and Soules, 1988; De Souza et al., 1998). A 3 month sample prevalence and incidence of LPD and anovulation of 48 and 79% respectively was observed (De Souza et al., 1998). In addition, abnormal ovarian function (LPD and anovulation) was consistently displayed by 33% of the exercising women and inconsistently displayed by 46% of exercising women. In other words, despite the fact that all of the women in the study had consistent and repeatable menstrual cycle lengths of ~27 days, almost half (42%) of the exercising women had inconsistent menstrual status from cycle to cycle; that is, intermittent presentations of ovulatory, LPD and anovulatory cycles. In contrast, 100% of the sedentary women had consistent menstrual status presentations from cycle to cycle, emphasizing the importance of monitoring more than one menstrual cycle in research studies (McNeely and Soules, 1988; De Souza et al., 1998). Figure 4 represents a hypothetical presentation of the varied menstrual cycle status one might observe in an exercising woman during a 1 year period.

**Detection of exercise-associated menstrual disturbances**

**Methods of detection**

Much of the data that has been collected in attempts to define menstrual status, length and regularity in athletic populations has been the product of survey, questionnaire and self-report data (Feicht et al., 1978; Dale et al., 1979; Schwartz et al., 1981; Sanborn et al., 1982; Carlberg et al., 1983). The use of other techniques to assess menstrual status includes hormonal measures made in blood, urine and saliva and is discussed below. The inherent difficulties and inaccuracies ascribed to recall and self-report are well documented. Significant errors in memory distortion can occur when assessing cycle regularity or irregularity that relies on recall of menstrual cycle length (Weller and Weller, 1998; Schneider et al., 2003). A review of the literature of large studies conducted on the distribution of menstrual cycle lengths across large groups of women of varying ages reveals a large range in what is considered ‘typical’ or ‘regular’. There is no accepted objective criterion for what constitutes regularity or irregularity (Weller and Weller, 1998). The commonly observed interval between menstrual cycles is 26–30, 24–32 and 24–35 days respectively (Wentz, 1998; Couchman and Hammond, 1994; Speroff et al., 1994). In studies of menstrual function in exercising women, the range of 26–32 days has frequently been used to denote ‘normal menstrual cycle length’ (Treloar et al., 1967; Loucks et al., 1989; De Souza et al., 1998). Adoption of this range as ‘normal’ is well within the findings of many larger epidemiological studies of healthy women in non-exercise-related studies, and remains consistent with many studies of female athletes in the literature. Self-reported cycle length may be useful if the shortcomings of such data are recognized, and cycles deemed to be ‘irregular’ are clearly outside commonly accepted ranges.

As a reflection of menstrual status and reproductive function, cycle length is not nearly as informative as actual endocrine measures of reproductive hormones. Even though anovulation, and inadequate luteal function have both been associated with both short (<21 days), and long (>35 days) cycle lengths (Harlow and Matanoski, 1991; Speroff et al., 1994; Waller et al., 1998), these abnormalities can occur when cycle length remains in the ‘normal range’.

**Amenorrhoea and oligomenorrhoea**

The clinical diagnosis of primary amenorrhoea or secondary amenorrhoea in an athlete requires a full evaluation by a physician (Speroff et al., 1994). Once pregnancy has been ruled out, other endocrinopathies associated with menstrual irregularity should be pursued, such as prolactin-secreting pituitary tumours, thyroid problems, polycystic ovarian syndrome, and premature ovarian failure (Speroff et al., 1994). A full examination, including a pelvic and physical examination, and appropriate laboratory analyses are required. In a research setting, it is important to establish a thorough reproductive hormone profile for assessing E2 exposure. This will require daily urine samples and measurements E2 and progesterone metabolites, and LH. In an oligomenorrhoeic athlete, an identical approach should be pursued.

**Luteal phase defects and anovulation**

Duration of luteal phase length is made by determining the interval between ovulation and menses where ovulation is assessed by the direct determination of the day of the LH surge, which is the technique of choice in many laboratories. The use of basal body temperature alone to determine the day of ovulation is inferior to the documentation of an LH surge (Jones, 1976;
Balasch and Vanrell, 1987; Jordan et al., 1994). The criterion of a ‘short’ luteal phase has been inconsistently presented in the infertility literature and has ranged from 9 to 11 days (Strott et al., 1970; Sherman and Korenman, 1974; Jones, 1976; Balasch and Vanrell, 1987; McNeely and Soules, 1988; Jordan et al., 1994). Sherman et al. (1974) and Strott et al. (1970) defined a luteal phase of <10 days as ‘short’ since both low peak levels of progesterone and decreased follicular FSH were observed in association with this shortened luteal phase length. In the exercise literature, variable criteria have been utilized but have included the use of that originally presented by Sherman et al. (1974) and Strott (1970). It is important to recognize that short luteal phases alone (without reduced progesterone) are not consistently linked to infertility in the clinical literature (Jones, 1976; Balasch and Vanrell, 1987; McNeely and Soules, 1988; Jordan et al., 1994) and therefore should not be considered a sensitive diagnostic criterion for LPD.

Used alone or in combination with luteal phase length, the determination of daily progesterone levels for at least one cycle, but preferably multiple cycles, has been recommended as the ‘gold’ standard for the identification of LPD for research purposes (Jones, 1976; Balasch and Vanrell, 1987; McNeely and Soules, 1988; Jordan et al., 1994). Other investigators have also noted considerable variability in menstrual cycles and have recommended that at least three consecutive cycles be monitored for an accurate assessment of menstrual status (McNeely and Soules, 1988). The use of single serum daily measurements is limited since daily blood sampling is overly invasive. Additionally, since progesterone is secreted in a pulsatile manner, significant variability in the range of 5–15 ng/ml of mid-luteal serum values for LPD has been reported (McNeely and Soules, 1988; Jordan et al., 1994). Less invasive strategies than daily blood sampling for measuring progesterone levels in a research setting can be accomplished using daily collections of urine or saliva. Criteria for an inadequate luteal phase are varied; however, Kesner et al. (1992) and Santoro et al. (1992) have suggested criteria for urinary pregnanediol 3-glucuronide (PDG). Achieving a critical level of 5 μg/mg Cr of PDG during the luteal phase or 4 days of values >3 μg/mg Cr of PDG during the luteal phase is suggested as the criterion for an adequate luteal phase (Kesner et al., 1992; Santoro et al., 1992). Clearly, investigators of menstrual status need to consistently apply such criteria.

The most accurate method to detect ovulation is ultrasound observation of follicular growth and rupture (Hata et al., 1983), and when not possible, measurements of mid-cycle LH concentrations combined with luteal phase reproductive steroid levels (Royston, 1983; Baird et al., 1991; Kassam et al., 1996; McConnell et al., 2002). Since ultrasound testing requires special equipment and expertise, and is costly, and obtaining blood samples across an entire menstrual cycle is prohibitive, researchers conducting large scale studies evaluating the menstrual cycle have turned to the measurement of urinary metabolites of LH, E₂ and progesterone (Royston, 1983; Baird et al., 1991; Kassam et al., 1996; McConnell et al., 2002). If measurement of estrone glucuronide (E1G) and PDG is undertaken prior to assaying LH, this can serve to reduce the required number of samples necessary to locate the LH peak and subsequently estimate the day of ovulation. If the cost of assaying LH is prohibitive, another option for researchers is to objectively determine the presence (or absence) of ovulation with the use of established algorithms (Royston, 1983; Baird et al., 1991; Kassam et al., 1996). Algorithms have been validated in large samples of eumenorrheic women in whom urinary measurements of E₂ and progesterone metabolites have been assayed. The use of objective algorithms developed in normally cycling women with classical hormone patterns has recently been validated in exercising women (McConnell et al., 2002). The latter approach reduces experimental bias inherent with visual inspection, and allows for consistent treatment of the data.

Aetiology of exercise-associated menstrual disturbances (EAMD) in athletes

It is believed that during times of chronic energy deficiency, a shift in metabolic fuels occurs that repartitions energy away from the costly processes of reproduction and towards the essential processes of cellular, locomotive and other life-sustaining metabolic functions (Wade et al., 1996). With respect to exercising women, eloquent short-term experiments by Loucks (Loucks and Callister, 1993; Loucks and Heath, 1994; Loucks and Thuma, 2003; Loucks et al., 1998) manipulating both dietary intake and energy expenditure have revealed a close correlation between energy availability and the modulation of the GnRH pulse generator. Additional evidence for the association between low energy availability and EAMD is found in studies reporting 400–700 calorie differences between reported food intake and estimates of energy expenditure (Williams, 1998) and cross-sectional studies of 24 h patterns of metabolic hormones and substrates that illustrate adaptive changes resembling those with prolonged undernutrition (Loucks et al., 1989; Laughlin and Yen, 1996). Studies in AA, combined with observational studies exposing metabolic signs of energy deficiency associated with subtle menstrual disturbances such as LPD and anovulation (De Souza et al., 2003), provide strong evidence that a hypometabolic state exists commensurate with EAMD. This concept is illustrated in Figure 5. This hypometabolic state includes reductions in resting metabolic rate, total T₃, leptin, insulin, glucose, and insulin-like growth factor binding protein (IGFBP)-3 and elevations in IGFBP-1, ghrelin, growth hormone and cortisol (Loucks and Callister, 1993; Loucks and Heath, 1994; Laughlin and Yen, 1996; Loucks and Thuma, 2003; De Souza et al., 2004a).

Support for a causal relationship between energy availability and menstrual cyclicity was recently provided by Williams et al. (2001a,b) who demonstrated that amenorrhoea in exercising monkeys could be reversed by increasing food intake while daily training was maintained. The restoration of ovulation in the amenorrhoeic monkeys exhibited a dose–response relationship with energy availability, as the numbers of days until ovulation occurred was significantly correlated with the amount of calories provided, such that monkeys that ate more calories recovered more quickly. The additional observation in this study that changes in circulating total T₃ (a marker of energy balance) was correlated with both the induction and reversal of amenorrhoea lends support to the idea that the suppression of reproductive function was linked with adaptive mechanisms to reduce energy expenditure (Williams et al., 2001b) when an imbalance was created by the increased energy cost of exercise in the face of inadequate supplementation of caloric intake.
While our understanding of the importance of low energy availability in the aetiology of EAMD has advanced, the mechanism by which a state of low energy availability is communicated to, and has direct effects on, the GnRH pulse generator remains to be determined. An earlier emphasis on the importance of body weight and or body fat per se (Frisch and Revelle, 1970; Frisch and McArthur, 1974) as the critical link between energy status and reproductive function has been questioned by findings in short-term studies in humans and animals, demonstrating that exercise-associated amenorrhea occurs at a range of body weights and percentage body fat (Sanborn et al., 1987), and close correlation with changes in LH pulsatility and energy availability (Loucks, 2003; Loucks and Thuma, 2003). Further, in the monkey model of EAMD, body weight showed no significant changes during the development of amenorrhea (Williams et al., 2001a). Given that body weight changes in response to a given energy surplus or deficit exhibit large inter-individual variability (Bouchard and Tremblay, 1997), other indicators of alterations in energy balance such as changes in metabolic hormones or substrates may be more likely candidates as key signals to GnRH neurons or other neurotransmitter...
systems that modulate GnRH. Indeed, a plethora of studies reviewed elsewhere (Wade et al., 1996) suggest the potential for numerous metabolic factors to directly or indirectly signal changes in peripheral energy status to the brain. Undoubtedly, the system exhibits redundancy, and a particular signal may be associated with the magnitude of, and/or time frame over which, an energy deficit is experienced. Other factors may include initial energy stores and whether a deficit is created by a reduction in food intake and/or by an increase in exercise. Data are conflicting regarding initial energy stores; Schneider and Wade (1989) have shown that lean hamsters are more susceptible to reproductive suppression caused by caloric restriction than are obese hamsters (Schneider and Wade, 1989), but Williams et al. (2001a) found no relationship between the time to development of amenorrhoea and initial body weight in the exercising monkeys. Additionally, the potential for synergistic effects with other factors that can suppress reproductive function, such as psychological stress, should be explored (Williams, 2003). Clearly, more work is needed to unravel the intricacies of the cross-talk between metabolism and reproduction.

Transition to amenorrhoea

A long-standing question in exercise and menstrual cycle research has been whether LPD in an athlete is likely to progress to a more severe menstrual disturbance such as anovulation or amenorrhoea, particularly if energy status is altered in an unfavourable direction. Similarly, it is important to know if anovulation progresses to amenorrhoea if energy status worsens. In other words, do menstrual disturbances fall along a continuum of energy availability, such that individuals transition between development of amenorrhoea and initial body weight in the exercising monkeys. Additionally, the potential for synergistic effects with other factors that can suppress reproductive function, such as psychological stress, should be explored (Williams, 2003). Clearly, more work is needed to unravel the intricacies of the cross-talk between metabolism and reproduction.

Recovery from amenorrhoea

Without moderating the volume or intensity of the exercise protocol in the monkey studies, Williams et al. (2001a) was successful in reversing the amenorrhoea over a range of 12–57 days in four monkeys simply by supplementing their energy intake ad libitum without moderating the exercise training regime at all. Body weight significantly increased and menses returned in all four monkeys. In the menstrual cycle immediately following amenorrhoea, progesterone levels were increased relative to the amenorrhoeic period, and consistent with ovulatory levels. However, these levels were lower than those in the cycle preceding amenorrhoea (Williams et al., 2001a). These data presumably suggest that during the transition from ovulatory cycles to amenorrhoea, LPD and anovulatory cycles associated with inadequate progesterone production, lengthening of the follicular phase and decreased LH are apparent. Also, it appears that LPD also occur during the transition back to ovulatory cycles from amenorrhoea. The concept of transitioning from ovulatory to anovulatory and LPD cycles is illustrated by the hypothetical menstrual cycles in Figure 4.

Bone health issues associated with EAMD

Bone loss and amenorrhoea

Despite the reported stimulatory effect that exercise has on bone, it is well documented that AA unequivocally suffer from reductions in BMD, particularly in the lumbar spine (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985; Tomten et al., 1998; Gremion et al., 2001; Csermely et al., 2002; Warren et al., 2002; Cobb et al., 2003). Even in oligomenorrhoeic athletes, very low BMD has been repeatedly reported (Tomten et al., 1998; Gremion et al., 2001; Csermely et al., 2002; Cobb et al., 2003). In one study of oligomenorrhoeic athletes, lumbar BMD was only 69% of that observed in an aged-matched cohort.
of menstruating women (Cobb et al., 2003). Bone loss observed in AA may be serious enough to result in osteoporotic fractures well before menopause including an increased risk of stress fractures, and fractures of the hip and spine (Carbon et al., 1990; Dugowson et al., 1991; Otis et al., 1997; Korpelainen et al., 2001). Deficits in BMD during the pre- or early menopausal period must, in fact, result from either the failure to attain peak BMD and/or as result of bone loss during the pre-menopausal years. Other key observations are that the duration of amenorrhea is inversely related to BMD, and the risk of bone loss is greatest early after the onset of amenorrhea (Beverly et al., 1991). BMD is associated with both current menstrual status, and one’s history of EAMD, as shown by Drinkwater et al. (1990). Bone loss in pre-menopausal women is also recognized as the failure to achieve peak bone mass. Peak bone mass appears to be a very good predictor of the rate of post-menopausal bone loss, and lower levels will lessen the time to increased risk of fracture (Ott, 1990; Matkovic et al., 1994; Kanis, 2002).

Establishing prevalence of bone loss in female athletes

One difficulty in establishing the prevalence of bone loss in female athletes is the choice of criteria utilized. The use of the appropriate clinical criterion, i.e. t-score or z-score, representing osteopenia or osteoporosis is dependent on age along with consideration of secondary risk factors, as recently recommended by the International Society of Clinical Densitometry (Khan et al., 2002; Khan and Syed, 2004). In athletes, bone loss more frequently meets the clinical criterion of osteopenia, not osteoporosis (Khan et al., 2002). Osteopenia is defined with a t-score in any region of interest of −1.0 to −2.5 which is 1 to 2.5 SD below the mean of that observed in young adults Kanis, 2002). Osteoporosis represents a t-score in any region that is >2.5 SD below the mean achieved in young adults. Osteopenia is associated with a 100% increase in the risk of fracture and is notably of long-term concern for the management of bone health in these individuals (Kanis, 2002; Khan et al., 2002). Osteoporosis represents an even greater risk of fractures (Kanis, 2002). The prevalence of osteopenia in AA is estimated to range from 1.4 to 50% in athletes (Drinkwater et al., 1984; Dugowson et al., 1991; Rutherford, 1993; Young et al., 1994; Lauder et al., 1999; Pettersson et al., 1999; Cobb et al., 2003). The prevalence of osteoporosis is lower (Lauder et al., 1999; Cobb et al., 2003).

Bone loss with anovulation and LPD

The potential for bone loss with less severe menstrual disturbances such as anovulation and LPD has been much more difficult to study due to the methodological difficulties encountered in characterizing these more subtle menstrual disturbances, and the limited studies available. Data reported by Prior et al. (1990) suggested that LPD and anovulatory cycles resulted in progressively more spinal bone loss (−4.2%) over a 1 year period in moderate and long-distance runners, and that luteal phases <9.9 days were associated with significant bone loss over 1 year in exercising women (Petit et al., 1999). Alternatively, in a cross-sectional study, De Souza et al. (1997) subsequently reported that BMD in exercising women with LPD were comparable to ovulatory sedentary women (De Souza et al., 1997). Winters et al. (1996) noted lower whole body calcium per kg of lean tissue and lumbar spine BMD in their runners compared to the active controls, although this represented only a trend toward significance. In the Women’s Health Reproductive Health Study, Waller et al. (1998) provided similar data in sedentary women with LPD. Whether or not these latter studies were sufficiently powered to detect small differences in BMD is debatable (Petit et al., 1998).

Hypoestrogenism and bone loss with EAMD

It has generally been accepted that chronic hypoestrogenism is a major cause of bone loss in women, regardless of age (Ott, 1990; Matkovic et al., 1994; Kanis, 2002). E2 plays an important role in promoting bone mass in adolescents and young adults and in maintaining bone mass in adult women. Several studies supporting a mechanistic role of E2 exist (Riggs et al., 1998, 2003; Jarvinen et al., 2003). It is therefore likely that hypoestrogenism in AA contributes to bone loss in a similar manner when compared to that observed in post-menopausal women (Cann et al., 1984; Drinkwater et al., 1984; Marcus et al., 1985). Supportive data are available in women with E2 deficiency associated with anorexia nervosa (Zipfel et al., 2001). Since exposure to E2, along with genetic and nutritional factors, determines peak bone mass (Ott, 1990; Matkovic et al., 1994; Kanis, 2002), it has been believed that a delay in menses related to exercise training may result in a lower than normal peak values.

The mechanism for bone loss with more subtle EAMD has also been linked to hypoestrogenism, although some support exists for a role for progesterone (Prior et al., 1990, 1994). Perhaps the most convincing data supporting the concept that subtle disturbances in ovarian steroids are associated with decreased BMD is the work of Sowers et al. (1998a,b), showing that pre-menopausal women aged 25–44 years who have subclinical decreases in E2 have decreased BMD and that they may have an impaired ability to maintain their bone mass, or achieve peak bone mass, depending on their age. Sowers et al. (1998a,b) also showed that those pre-menopausal women with the lowest BMD also had the most disturbances in sex steroids during the luteal phase. In studies of exercising women documenting both anovulation and LPD with daily urine metabolites of E1C and PDG, evidence for decreased E2 exposure has been reported (Winters et al., 1996; De Souza et al., 1997). Although reductions in BMD were not observed, De Souza et al. (1997) found significantly lower E2 levels in the follicular phase on days 2–12 in the LPD runners, and lower E2 levels during the luteal phase, as assessed by area under the curve. Even in the ovulatory exercising women without LPD in that study, E2 levels were lower during the early follicular phase on days 2–5, indicating that overall E2 exposure may be inadequate in some cases. If future studies that are powered sufficiently to examine bone outcomes...
suggest that mild reductions in $E_2$ can negatively impact bone, it may explain why some normally menstruating women, i.e. those observed by Cobb et al. (2003), also have osteopenia. Whether a specific threshold for $E_2$ exposure is critical for bone health remains undefined in the literature.

**Caloric restriction and bone loss with EAMD**

In addition to considering the degree of hypoestrogenism as a predictor of bone loss in women with EAMD, the impact of nutritional factors, such as micronutrient deficits, caloric restriction and chronic undernutrition should also be pursued as potential causes of diminished BMD. The effects of caloric restriction may work indirectly through energy deficiency-induced decreases in $E_2$ and/or directly through micronutrient deficiencies (Miller, 2003) or reductions in bone trophic factors such as IGF-I and possibly leptin (Flier, 2002; Goldstone et al., 2002; Grinspoon et al., 1995a,b, 1996, 1997; Khosla, 2002; Takeda et al., 2002; Miller, 2003). Recent studies have documented the co-existence of hypoleptinaemia and osteopenia (Matejek et al., 1999; Warren et al., 1999; Kaufman et al., 2002). Since a significant link between energy deficiency and EAMD exists (Bullen et al., 1985; Williams et al., 2001), and since studies have clearly documented decreases in key endocrine parameters important for bone in athletes with EAMD (Laughlin and Yen, 1996; De Souza et al., 2003) it is plausible that caloric restriction has a direct impact on bone health in female athletes. Previous studies concluding that hypoestrogenism contributes substantially to bone loss in female athletes most likely neglected to consider the potential for an additional and independent impact of caloric restriction on bone. Evidence in support of this includes several observations that oral contraceptive use in hypothalamic amenorrhoea associated with anorexia, exercise or other causes is not associated with complete bone recovery (Hergenroeder et al., 1997; Zipfel et al., 2001; Grinspoon et al., 2003). Similarly, recovery of menses in former AA does not result in complete recovery of BMD (Drinkwater et al., 1986; Jonnavithula et al., 1993; Keen and Drinkwater, 1997). Further, weight loss, caloric restriction and restrained eating have been associated with bone loss in humans and animals (Lee et al., 1993; Barr et al., 1994; Ramsdale and Bassey, 1994; Shaptes et al., 2001; Talbott et al., 2001). In humans, a 10% decrease in body weight has been shown to result in a 1–2% loss in BMD (Compston et al., 1992; Hyldestrup et al., 1993; Andersen et al., 1997).

Recent studies have utilized biochemical markers of bone turnover to assess the effects of energy deficiency associated with exercise on bone. Zanker and Swaine (1998) have shown that markers of bone formation are suppressed in AA, while markers of bone resorption are unchanged. Because markers of formation appear to be responsive to energy deficiency, and markers of bone resorption indicate inadequate $E_2$, this latter finding supports a stronger contribution of energy deficiency to bone loss with amenorrhoea. Recently, Ihle and Loucks (2004) have eloquently demonstrated the relationship between varying levels of an energy deficit and markers of bone turnover in exercising women. An intriguing dose–response relationship between bone markers and reproductive and metabolic hormones was observed, such that at moderate volumes of energy restriction, markers of bone formation were suppressed, whereas severe volumes of energy restriction were required before bone resorption was increased. The increase in bone resorption was not observed; however, until a severe degree of energy restriction was imposed and associated with a suppression of serum $E_2$ by 18%. The markers of bone formation, on the other hand, were decreased in a manner very similar to that observed for several metabolic hormones, including $T_3$, insulin and IGF-I, in conditions of a moderate degree of energy restriction. These data demonstrate a plausible $E_2$-independent pathway whereby bone turnover, and specifically bone formation, is suppressed and thus may contribute to decreased BMD in athletes experiencing chronic energy deficiency (Ihle and Loucks 2004). These findings may explain why reductions in BMD might be expected in eumenorrhoeic women with LPD and anovulation who have slight metabolic hormone alterations indicative of a moderate energy deficit, but do not have severe reductions in $E_2$ (De Souza et al., 2003).

**Hypoestrogenism, EAMD and cardiovascular disease risk**

Recent findings suggest that chronic hypoestrogenic states may predispose athletic women to early cardiovascular disease. Clinically, one of the earliest indicators of cardiovascular disease is a decrease in endothelial function that is evident decades before overt coronary artery disease is present (Celermajer, 1997; Luscher and Barton, 1997; Schachinger et al., 2000). Atherosclerotic disease progression and adverse cardiovascular events have both been shown to be associated with a decrease in endothelial function (Celermajer, 1997; Luscher and Barton, 1997; Schachinger et al., 2000). In addition, physical changes to the endothelium and the availability of nitric oxide are also modified commensurately with atherosclerotic disease (Celermajer, 1997). Since nitric oxide is a major regulator of basal vascular tone and plays a key role in vasodilation, factors that impair nitric oxide production, such as hypoestrogenism and dysfunctional lipid metabolism, can be associated with cardiovascular disease (Steinberg, 1987; Celermajer, 1997; Luscher and Barton, 1997; Mendelsohn and Karas, 1999; Schachinger et al., 2000).

**Lipids, EAMD and hypoestrogenism**

Approximately 25–50% of the potential $E_2$ cardioprotective effect is associated with its beneficial impact on blood lipids (Steinberg, 1987). Elevation of lipoprotein levels > 160 mg/dl is recognized as an independent risk factor for cardiovascular disease (Steinberg, 1987). Low density lipoprotein (LDL), especially following oxidative modification within the vessel wall, may affect endothelial nitric oxide production and bioactivity, thereby causing endothelial dysfunction (Mendelsohn and Karas, 1999). In menopausal women, decreased $E_2$ levels are linked with alterations in lipid metabolism, including increases in total cholesterol, low density lipoprotein (LDL) and triglyceride, and decreased high density lipoprotein (HDL) (O’Connell and Genest, 2001). In younger women, a similar paradigm has been observed in clinical conditions associated with hypoestrogenism, including anorexia nervosa (Balika et al., 1990). AA represent an population at risk for these cardiovascular-related clinical concerns, given their chronic exposure to hypoestrogenism. Despite the exercise training in these women, AA have negative modifications in measures of lipid metabolism in
some, but not all, reports (Lamon-Fava et al., 1989; Friday et al., 1993). Elevated levels of triglycerides, cholesterol and LDL in particular have been reported in AA compared to menstruating athletes (Lamon-Fava et al., 1989; Friday et al., 1993). However, other studies have failed to observe these findings (Baer, 1999), but have frequently observed a tendency for the AA to possess a less favourable lipid profile, particularly an increased LDL level. The long-term implications of EAMD on lipid metabolism have yet to be assessed, especially across a range of subclinical levels of hypoestrogenism.

Vascular dysfunction and hypoestrogenism
Hypoestrogenism is associated with endothelium-dependent dysfunction (Guetta et al., 1997; Mendelsohn and Karas, 1999). E2 plays a significant role in endothelial-dependent blood flow via nitric oxide (Guetta et al., 1997; Mendelsohn and Karas, 1999). The mechanism of action leading to deterioration in endothelial function is likely through the E2-mediated nitric oxide pathway, which is known to be critical in vascular control (Guetta et al., 1997; Mendelsohn and Karas, 1999). Genomic and non-genomic effects of E2 have suggested that E2 influences vascular endothelial release of nitric oxide through actions that enhance the bioavailability of nitric oxide production (Guetta et al., 1997; Mendelsohn and Karas, 1999). Coincident with declining E2 levels, a study by Celermajer et al. (1994) has shown a reduction in endothelial-dependent vasodilation, 3 months after natural menopause. Impaired endothelial function has also been demonstrated 1 week after surgical menopause (Ohmichi et al., 2003). Given these rapid reductions in endothelial-dependent vasodilation following both surgical and natural menopause, it is logical to question the impact of clinical and subclinical levels of hypoestrogenism in young physically active women.

To date, only one study has examined the impact of athletic amenorrhoea on endothelial function. Zeni-Hoch et al. (2003) examined brachial artery flow-mediated dilation (endothelium-dependent) in AA and compared them to women with oligomenorrhoea and age-matched controls. Zeni-Hoch et al. (2003) reported that endothelial function was significantly lower (80%) in AA, compared to the menstruating athletes. In contrast, endothelium-independent dilation to nitroglycerine was not different between the groups. Recent data from our laboratory (O’Donnell et al., 2004) using venous occlusion plethysmography to examine lower leg blood flow has demonstrated lower resting (2.2 ± 0.1 versus 4.8 ± 0.4, P < 0.001) and peak ischaemic (42.8 ± 2.1 versus 52.9 ± 2.0; P = 0.004) blood flow responses (ml/100 ml/min), i.e. reduced resting and maximal lower leg blood flow in the order of 100% decreased at rest and 28% decreased at maximal levels, when AA are compared to age-matched eumenorrhoeic active women. In addition, in a similar AA group that scored in the osteopenic range for bone density in the lumber spine, blood flow responses were also compromised (De Souza et al., 2004b). The AA in this study also had lower resting supine heart rate and supine resting systolic blood pressure, compared to their eumenorrhoeic counterparts. Alarming, the magnitude of impaired endothelium-dependent vasodilation observed in the AA was comparable to data previously reported in post-menopausal women, and older (60 ± 2 years) coronary artery-diseased patients, after a similar flow-mediated stimulus (Celermajer et al., 1992; Blumel et al., 2003). Stacey et al. (1998) reported lower nitric oxide metabolite levels in AA compared to eumenorrhoeic athletes and controls, suggested that these events are likely mediated by altered nitric oxide metabolism. These findings confirm altered flow-mediated endothelial-dependent vasodilation. Lower resting heart rate and systolic blood pressure decrease in AA, may indicate altered autonomic regulation, similar to that seen in anorexia nervosa patients (O’Donnell et al., 2004). Thus, these findings imply that despite participating in regular physical exercise, chronic hypoestrogenic states may predispose young athletic women to premature cardiovascular disease. Moreover, these findings represent an additional health paradigm associated with the hypoestrogenism coincident with the female athlete triad and suggest that hypoestrogenism in AA may lead to deleterious cardiovascular outcomes. Certainly, further evaluation is warranted.

Summary and conclusions
Energy deficiency in female athletes and active women can give rise to hypoestrogenism and is associated with the female athlete triad. The triad is a serious medical condition that signals the existence or co-existence of disordered eating, menstrual disturbances, and premature bone loss or the failure to achieve peak bone mass. Although large numbers of studies have not yet been conducted to establish the prevalence of the co-existence of all three aspects of this condition, existing data on the prevalence and physiological links between all combinations of one or two of the three conditions warrants action to prevent future occurrences and to treat existing cases. Furthermore, research on the subclinical expressions of each condition, and the interrelationships between these conditions, has expanded our definition of the triad to include less severe forms of disordered eating, subtle menstrual disturbances, and lesser degrees of bone loss. The observation that the triad can be found outside the athletic arena in recreationally active women demonstrates a need to broaden surveillance to include a larger proportion of young women. The aetiology of menstrual disturbances associated with the triad is causally linked to low energy availability, and as such there is no justification for fears that exercise itself is unhealthy for women. Physiological studies identifying candidate metabolic signals will continue to inform us of potential biomarkers of menstrual disturbances, and other studies will undoubtedly increase our knowledge of the threshold of energy availability below which menstrual disturbances occur. The hypoestrogenism associated with the range of menstrual disturbances factors heavily into the effects on bone and vascular health. New research in these areas has broadened the perspective of the triad to include the potential for cardiovascular disease and has heightened concern regarding the potential for bone loss in women with anovulation and perhaps even LPD. Future work should further explore these areas and carefully assess the wider implications that may impact women across the lifespan.

References


Sequelaes of energy deficiency and hypoestrogenism

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