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Energy availability and its relationship to salivary progesterone levels in elite adolescent aesthetic athletes

by

Karen Joanne Reading ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

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Karen Reading

Karen Joanne Reading
7625-107 St.
Edmonton, Alberta
T6C 4X6

January 28th, 1999

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ***Energy availability and its' relationship to salivary progesterone levels in elite adolescent aesthetic athletes*** submitted by **Karen Joanne Reading** in partial fulfillment of the requirements for the degree of Master of Science in *Nutrition and Metabolism.*

Linda J. McCargar, PhD
Supervisor

Vicki Harber, PhD

Catherine J. Field, PhD

Approved by Committee: January 24 1999

ABSTRACT

The purpose of the present study was to examine the role a negative energy balance plays in the development of menstrual abnormalities and whether resting energy expenditure is reduced in order to compensate for the apparent energy deficit in elite adolescent aesthetic athletes. Three groups of female adolescents (age 15 to 18) were studied; ten oligo/amenorrheic athletes, eleven eumenorrheic athletes and eight non-athlete controls. Components of energy balance, body weight/composition, dietary restraint, pubertal maturation and luteal phase salivary progesterone levels were compared. The two athlete groups were similar in all measures except luteal phase progesterone levels. The oligo/amenorrheic athletes had a significantly later pubertal development than the controls and lower luteal phase progesterone levels than the other two groups. Although the oligo/amenorrheic athletes were in a negative energy balance, it was not significantly lower than the other two groups and REE was not depressed. In conclusion, the present study lends no support for reduced REE as a mechanism of energy conservation. Luteal phase progesterone levels appear to be related to the stage of pubertal development and an immature reproductive hypothalamic-pituitary-ovarian axis, which is characteristic of adolescence.

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
AN	Anorexia nervosa
ATP	Adenosine triphosphate
BBT	Basal body temperature
BN	Bulimia nervosa
CRH	Corticotropin releasing hormone
DEBQR	Dutch Eating Behaviour Questionnaire
FT_3	Free triiodothyronine
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
IBW	Ideal body weight
kcal	Kilocalories
kcal/day	Kilocalories per day
kcal/kg	Kilocalories per kilogram body weight
kilopond	Kilopond
LBM	Lean body mass
LH	Luteinizing hormone
MET	Metabolic
PAR-Q	Physical activity readiness questionnaire
REE	Resting energy expenditure (kcal/day)
RMR	Resting metabolic rate (kcal/hour)
RQ	Respiratory quotient (VCO_2/VO_2)
r-T_3	Reverse-triiodothyronine
T_3	Triiodothyronine
T_4	Thyroxine
TEE	Total energy expenditure
TEF	Thermic effect of food
VLCD	Very low calorie diet
VO_2 Max	Maximal oxygen uptake

CHAPTER ONE
INTRODUCTION

I. Overview

Menstrual abnormalities are common in adolescents as puberty is characterized by endocrine changes that reflect an immature hypothalamic-pituitary-ovarian axis. Female athletes participating in aesthetic sports such as dance, gymnastics, diving, figure skating and synchronized swimming, all of which place an emphasis on thinness are even more susceptible to menstrual abnormalities (Shangold & Miram, 1993). Epidemiological studies have linked menstrual disturbances to the stress of physical training, weight loss, low body fat and the energy draining effect of intense physical exercise (Loucks & Horvath, 1985). Menstrual abnormalities in female athletes are typically associated with long hours of daily intensive training with an emphasis on a thin physique. This is often a result of inadequate nutritional intake for the level of activity, leaving the body in a negative energy status. Studies that have measured energy intakes of female athletes report caloric intakes well below estimated energy expenditure especially in sports requiring a low body weight (Beals & Manroe, 1994). The negative energy balance theory has recently become one of the accepted theories of menstrual abnormality; as the disruption of menstrual function is suggested to be an energy conserving adaptation to energy deficiency (Warren, 1973, Wade & Schneider, 1996, Loucks & Heath, 1998, Wilmore, 1992). Some researchers believe that in response to an energy deficit, the essential functions of survival are maintained at the expense of other activities that can be delayed such as reproduction, growth and adipose tissue storage.

Female athletes, in particular athletes involved in aesthetic sports are under significant pressure to achieve and maintain a low body weight. Not only do these athletes face the sociocultural pressures to conform to a thin physique, they face the demands of their sport placed on them from coaches and judges. The pressure to attain a low body weight may lead to potentially harmful patterns of restrictive eating in

participants of aesthetic sports. It has been reported that many athletes practice weight control methods similar to those reported in persons with eating disorders (Sundgot-Borgen, 1993). Nutritional status is a major concern for athletes practicing strict weight control methods.

Many investigators have shown that athletes have the ability to maintain normal weight despite a negative energy balance (Myerson et al. 1991, Deuster et al. 1986). Evidence exists to suggest that sustained insufficient caloric intake and intense exercise deplete energy stores, stimulating the body to increase food efficiency and lower metabolic rate (Myerson et al. 1991). Chronic dieting and restrictive eating with or without exercise is also suggested to play a role in the conservation of energy. Chronic energy restriction has been shown to produce a decrease in basal metabolic rate from 15 to 30 percent (Bray, 1969, Warwick & Garrow, 1981).

II. Justification for the Study

Researchers have demonstrated that about one-third of menstrual cycles in the adolescent years are anovulatory and this finding may be maintained up to 20 to 25 years of age (Vuorento & Huhtanieni, 1992). Anovulatory cycles are believed to be higher in aesthetic athletes since exercise and dietary restriction has been suggested to modulate female reproductive function. Inadequate energy may first lead to a reduction in luteal progesterone levels, then occasional and frequent anovulatory cycles, shortened cycle length, oligomenorrhea and finally amenorrhea with increasing levels of physical activity (For definition of terms, refer to Definition of Terms and Concepts section). Oligomenorrhea and amenorrhea are accompanied by low levels of estradiol and progesterone. Low levels of estradiol during adolescence may be associated with a lower rate of bone mineral accretion leading to decreased peak bone mass, a risk factor for developing osteoporosis in menopause or even premature osteoporosis. Clinical evidence has also linked luteal phase defects with reduced fecundity, the ability to reproduce (Ellison, 1993).

It is still uncertain why certain individuals are more susceptible to athletic amenorrhea than others. Recent research suggests amenorrheic females may have

different energy intakes and nutritional status than females with normal menstrual cycles (Wilmore et al., 1992, Marcus et al., 1985, Lloyd et al., 1987, Snow et al., 1990). There are limited data directly linking abnormalities of the menstrual cycle to a negative energy balance and restrained eating in adolescent aesthetic athletes and minimal data on energy balance in young athletes. It is anticipated that the effects of an energy deficit in athletes involved in aesthetic sports will be greater as weight control is an issue for many of these athletes. Some athletes may have more difficulty maintaining these unrealistic weight goals as many aesthetic sports are predominantly anaerobic in nature and include various components of training requiring less energy expenditure (ie. choreography).

The hormonal profile of oligomenorrheic and amenorrheic athletes includes a suppression of the ovarian sex hormones, estradiol and progesterone, along with elevated cortisol levels (DeSouza & Metzger, 1991). Chronic alterations of these hormones have potential adverse health effects on the function of the reproductive system. One particular triad of disorders termed the "Female Athlete Triad" is specific to female athletes participating in aesthetic sports and endurance runners. The triad begins with pressure to attain a specific body weight, which may lead to the development of an eating disorder. With the development of an eating disorder, amenorrhea is a common side effect. The reduced level of estradiol production associated with amenorrhea has detrimental effects on bone mineral status, increasing the risk of fracture and premature osteoporosis. This increased risk of fracture is a concern during the athletic career of the athlete, as well as a concern after menopause. The high incidence of eating disorders, menstrual dysfunction and use of pathogenic weight control practices reported by athletes involved in aesthetic sports suggests that they do not meet the energy demands of their highly active lifestyles.

III. Purpose

The purpose of the present study was to examine the hypothesis that a negative energy balance assists in the reduction in luteal progesterone levels and resting energy expenditure (REE) to compensate for the apparent energy deficit in adolescent athletes participating in aesthetic sports. Three groups of female adolescents were used to test this hypothesis; oligo/amenorrheic aesthetic athletes, eumenorrheic aesthetic athletes and eumenorrheic non-athlete controls.

IV. Study Objectives

1. To assess menstrual cycle status in adolescent aesthetic athletes versus non-athletes by measuring salivary luteal progesterone levels.
2. To assess the physiological and lifestyle factors associated with menstrual cycle status including body composition, energy expenditure, energy intake and restrained eating.
3. To determine if a negative energy is associated with a decreased REE and decreased luteal phase salivary progesterone levels.
4. To determine if restrained eating is associated with a decreased REE and decreased luteal phase salivary progesterone levels.

V. Independent and Dependent Variables

Independent Variable

1. A negative energy balance, as defined as a deficit of energy intake in relation to energy expenditure.

Dependent Variables

1. Luteal phase salivary progesterone profile over a one-month cycle.
2. Body composition (fat mass).
3. Resting energy expenditure.

VI. Research Hypotheses

1. Luteal phase salivary progesterone concentrations will be lower among the oligo/amenorrheic athletes compared to the eumenorrheic athletes and non-athlete controls.
2. The physiological and lifestyle factors will be different among the three study groups. Oligo/amenorrheic athletes will have a smaller sum of skinfolds (used an indirect assessment of body fat), and a reduced REE compared to eumenorrheic athletes and non-athlete controls. Dietary restraint and negative energy balance will be greater in oligo/amenorrheic athletes in comparison to eumenorrheic athletes and non-athlete controls.
3. A negative energy balance will be associated with a reduced REE and lower luteal phase progesterone levels.
4. Greater dietary restraint scores will be associated with a reduced REE and lower luteal phase progesterone levels.

VII. Definition of Terms and Concepts

1. The Menstrual Cycle

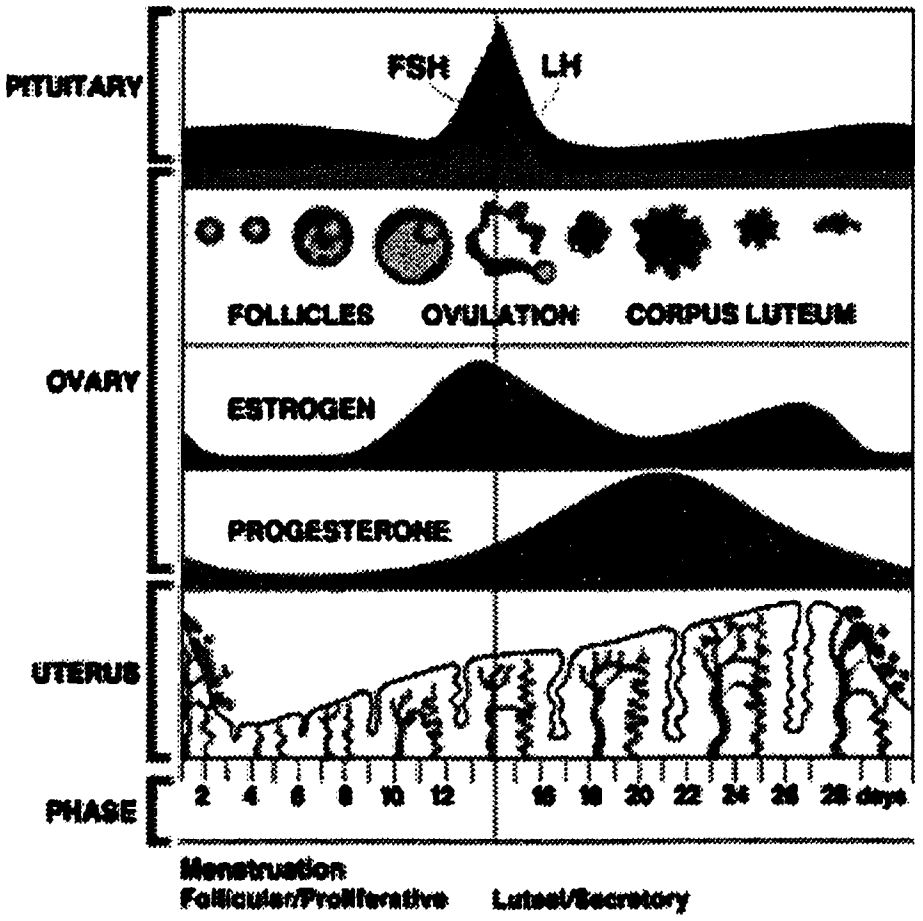
The female reproductive system undergoes a series of regular cyclic changes termed the menstrual cycle. Normal menstrual function results from the interaction of the hypothalamus, pituitary and the ovaries. External stimuli and stressors affect the system via hormonal signals to the hypothalamus, which produces the peptide gonadotropin releasing hormone (GnRH). GnRH acts as a releasing factor for pituitary hormone synthesis. The hypothalamus releases GnRH in pulses which stimulates the production and pulsatile secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. The normal menstrual cycle is typically 25 to 35 days with a median cycle length of 28 days (Lobo, 1991). The menstrual cycle consists of two constantly alternating phases, the follicular phase and the luteal phase.

The first half of the menstrual cycle called the follicular phase or proliferative phase is dominated by the presence of maturing follicles (Refer to Figure 1.1). The follicle operates in the first half of the cycle to produce a mature egg ready for ovulation. The maturing ovarian follicle secretes estradiol under the influence of FSH and LH and estradiol promotes the growth of the endometrial lining. The follicular phase is variable in length but lasts on average 15.1 days (Otis, 1992). It is the follicular phase that accounts for the individual difference in total menstrual cycle length (Otis, 1992).

The luteal or secretory phase of the menstrual cycle reflects the functional lifespan of the corpus luteum. It is the second half of the cycle and is defined as the interval from ovulation to the onset of menstrual bleeding. The luteal phase starts with the onset of the mid-cycle LH surge, which proceeds follicle rupture by approximately 24 hours (Hillier & Wickings, 1985). The chain of events is as follows: Rising levels of estradiol from the follicle signal the pituitary that a follicle is maturing. The pituitary then releases a burst of LH, which stimulates ovulation of the mature follicle and an egg is released. The follicular cells are transformed into the corpus luteum, which secretes progesterone. If fertilization does not occur, luteal function declines with decreasing

menstruation occurs (Refer to Figure 1.1). The onset of menstruation is the end of the luteal phase. The luteal phase is relatively constant and averages 14 plus or minus two days (Otis, 1992).

Figure 1.1. Hormonal Events During the Menstrual Cycle

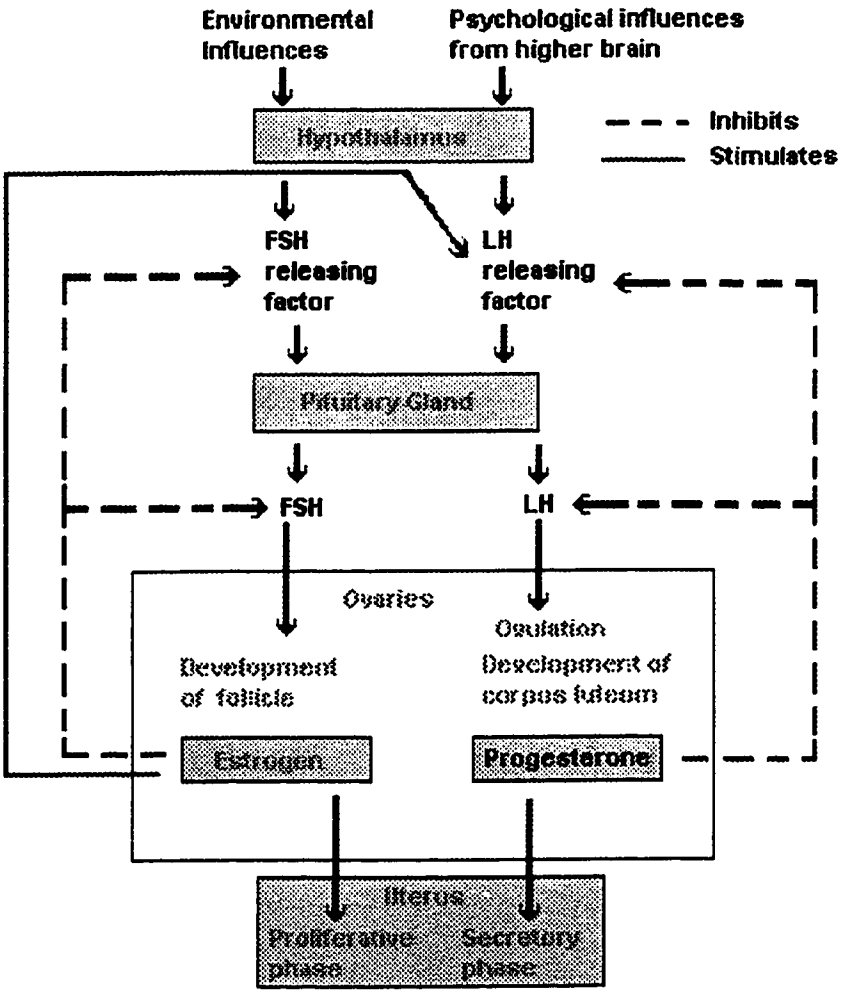


From: Merck & Co., Inc., "Normal physiology of the reproductive system: Hormonal events during the menstrual cycle." Copyright 1997, Merck & Co.,, Inc., Whitehouse Station, NJ, USA. (Sept. 1998).

2. The Hypothalamic-Pituitary-Ovarian Axis

The hypothalamic-pituitary-ovarian axis is the term used to describe the neuronal and hormonal interactions that act to regulate the menstrual cycle. Ovarian function is regulated by the pituitary gonadotropins, FSH and LH whose secretion is dependent on GnRH which is synthesized in the arcuate nucleus of the hypothalamus (Refer to Figure 1.2). The secretion of GnRH from the hypothalamus is regulated by the ovarian steroids, estradiol and progesterone, as well as by internal and external neural stimuli from the central nervous system (CNS) (Goodman 1988).

Figure 1.2. Hormone Feedback Control: The Hypothalamic-Pituitary-Ovarian Axis



Reproduced with permission from Husky RJ: Husky, Robert J. "Hormone Feedback Controls in the Female Reproductive Cycle." Nov. 1995. < http://www.wsrv.clas.virginia.edu/~rjh9u/menscycl.html. (Aug. 1998).

3. Maturation of the Hypothalamic-Pituitary-Ovarian (HPO) Axis

Ovulatory menstrual cycles are associated with the maturation of the HPO axis. During childhood (age 4 -10 years) the levels of gonadotropins and steroid hormones are low. Administration of GnRH to a prepubertal girl can induce ovulation, suggesting the ovaries are fully developed and capable of stimulation (Yen, 1991). An increased production of adrenal androgens is the first endocrine change associated with puberty, although the mechanism and role of the increased production is unknown. The increase in dehydroepiandrosterone (DHEA) suggests the adrenal cortex may play a role in the process leasing to puberty. At the onset of puberty an unknown stimuli is responsible for the pituitary release of the gonadotropins. The prepubertal gonadotropins are secreted only nocturnally, and are low in amplitude and pulse frequency in comparison to pubertal secretion levels. As puberty progresses the pulse frequency and amplitude of the gonadotropins increase and the diurnal variation diminishes. Rising concentration of FSH in early puberty stimulates the ovaries to increase the production of estradiol. The arcuate nucleus reduces its sensitivity to the sex steroids, resulting in an increase in pulsatile GnRH release. The rising level of estradiol production and reduced sensitivity of the arcuate nucleus stimulates the development of the secondary sexual characteristics. Estradiol stimulates endometrial proliferation, and gradually, over a period of about two years, endometrial proliferation is enough to initiate menarche (Goodman, 1988). Maturation of the HPO axis does not occur until estradiol production from a mature follicle reaches a critical level and stimulates the first LH surge from the pituitary resulting in ovulation (Goldfein & Monroe, 1997)

4. The Hormonal Profile of the Adolescent Menstrual Cycle

Literature describing the hormonal profile of the adolescent postmenarche is inconsistent and much still remains unknown as puberty is characterized by endocrine profiles that are both variable and unpredictable. The hormonal changes that occur during adolescence extend over several years both prior to and after menarche (Apter & Vihko, 1977). Menarche is typically characterized by irregular and anovulatory cycles and a still immature HPO axis and it takes several years post-menarche for a regular

pattern of menstrual cycles to occur. The incidence of ovulatory cycles may vary between ten and ninety percent of menstrual cycles in the first year of menarche, despite a midcycle LH surge (Goldein & Monroe, 1997). The first menstrual cycle after menarche is usually the longest, and cycle length gradually declines over the first three cycles (Apter et al., 1978). Previous findings have shown that the majority of cycles in girls with a gynecological age under 2 years are anovulatory and luteal phase progesterone levels have been reported to be low or undetectable until 1 to 2 years postmenarche (Winter & Faiman, 1973, Read et al., 1984, Vuorento et al., 1989). Several reports suggest that the incidence of ovulatory cycles reaches ninety percent five years after menarche (Apter et al., 1977, Vuorento & Huhtaniemi, 1992). Menstrual cycle abnormalities such as short luteal phase lengths and long follicular phase lengths are common in adolescents (Apter et al., 1978). In cycles with long follicular phases, a slow rise or deficiency of FSH during the follicular phase is usually the cause which results in a diminished follicular development and a subsequent inadequate corpus luteum function and low progesterone concentrations (Apter et al., 1978, 1980, Strott et al., 1970).

Female gonadotropin patterns are correlated with advancing pubertal stage. FSH levels plateau after midpuberty whereas LH levels gradually rise throughout puberty. Serum estradiol levels reflect the increased gonadotropin production that occurs with advancing puberty (Lobo, 1991). Adrenal hormones may also play a role in puberty as testosterone has been found to be higher in anovulatory cycles and it appears to diminish with advancing puberty during the luteal phase (Apter & Vihko, 1977, Apter et al., 1978). Thus, the hormonal pattern of adolescent menstrual cycles is far from uniform, which makes it difficult to determine normal from abnormal.

5. Functions of the Ovarian Hormones: Estradiol and Progesterone

Estrogen has a variety of critical functions at the level of the ovary in addition to multiple systemic effects (Yen, 1991). Estradiol is required for the normal maturation of the female body by its stimulatory effect on the vagina, uterus, uterine tubes at puberty, as well as development of the endometrial lining. Estradiol is responsible for

the pubertal growth spurt and the closing of the epiphyses of the long bones during puberty (Goldfein & Monroe, 1997). Estradiol plays an important role in the secretion of the gonadotropins by exerting both negative and positive feedback on the pituitary. (Goodman, 1988). Estradiol also have a number of metabolic effects, including reduced motility of the bowel, enhanced coagulability of blood, decreased adipose tissue oxidation of ketones and increased synthesis of triglycerides (Goldfein & Monroe, 1997). Estradiol alters plasma lipid levels in a favourable manner (increases high density lipoproteins, decreases low density lipoproteins). Estradiol also has a protective effect on the resorption of bone by antagonizing the effects of parathyroid hormone.

Like estradiol, progesterone plays a role in glandular development of the breasts and endometrium. Progesterone's primary role is the maintenance of early pregnancy, as it is responsible for the preparation of the blastocyte for successful implantation (Goldfein & Monroe, 1997). Progesterone also exhibits metabolic effects in other tissues and organs, producing changes in carbohydrate, protein and lipid metabolism (Goldfein & Monroe, 1997).

6. Menstrual Status Definitions

Eumenorrhea is the term used to describe regular menstrual cycles with cycle intervals of 25 to 35 days (Goldfein & Monroe 1997).

Oligomenorrhea refers to inconsistent or irregular menstrual cycles with cycle lengths exceeding 35 days (Goldfein & Monroe, 1997).

Amenorrhea is classified into primary and secondary. Primary amenorrhea is the delay of menarche beyond age 16 (Loucks & Horvath, 1985). Secondary amenorrhea is defined as the absence of menses in women who were previously cyclic. It is the absence of 3 to 12 consecutive menstrual cycles with cycle intervals longer than 90 days (Yen & Jaffe, 1991, Loucks & Horvath, 1985). Amenorrhea is further classified according to the site of defect. For example, hypothalamic amenorrhea is defined as a disruption of the neuronal mechanisms that regulate the hypothalamus secretion of GnRH (Loucks & Horvath 1985).

7. Negative Energy Balance

A negative energy balance is defined as inadequate energy intake relative to total energy expenditure (Thompson, 1998).

8. Total Energy Expenditure

Total energy expenditure includes the energy expended by the body for resting energy expenditure, thermic effect of food, and for physical activity (Whitney, Hamilton, Rolfes, 1996).

9. Resting Energy Expenditure (REE)

REE is defined as the sum the body's tissue cells' activity under steady-state conditions. REE is expressed as the rate of heat production or oxygen consumption related to some unit of body size (Weir, 1949). REE represents 50 to 70 percent of the total daily energy expenditure, and includes the energy required to maintain vital body functions at rest.

10. Restrained Eating

Restrained eating is defined as limiting the amount of food that is eaten, and consciously eating less than desired (Ruderman 1985).

11. Aesthetic Athletes

Aesthetic athletes are defined as athletes' participating in a sport which emphasizes leanness and is judged on the aesthetic appearance of the body while performing. Such sports include gymnastics, figure skating, diving, synchronized swimming and ballet dance (Adapted from Beals & Manore, 1994).

12. Physical Activity

Physical activity is defined as "any bodily movement produced by skeletal muscles that results in energy expenditure" (Caspesen et al. 1985, p. 126).

13. Maximal Oxygen Uptake (VO_2 Max)

VO_2 max. is defined as the maximum volume of oxygen consumed per minute during aerobic exercise. VO_2 max is considered the single best estimate of cardiorespiratory capacity (McArdle, Katch, Katch, 1996).

14. Anorexia Nervosa (AN)

Anorexia nervosa is a clinical eating disorder characterized by a refusal to maintain body weight over a minimal level considered normal for age and height, a distorted body image, an intense fear of fatness or gaining weight while being underweight and amenorrhea (American Psychiatric Association, 1994).

15. Bulimia Nervosa (BN)

Bulimia nervosa is a clinical eating disorder characterized by recurrent episodes of binge eating, a feeling of lack of control over eating during bingeing, purging behaviour including self-induced vomiting and/or use of laxatives or diuretics, strict dieting or fasting, or vigorous exercise to prevent weight gain, and persistent over-concern with body shape and weight (American Psychiatric Association, 1994).

16. Eating Disorders, Not Otherwise Specified (EDNOS)

Eating disorders, not otherwise specified are defined as a classification of clinical eating disorders that do not meet the strict criteria for AN or BN (American Psychiatric Association, 1994).

CHAPTER TWO
LITERATURE REVIEW

I. Introduction

Menstrual irregularities are common in adolescents and are more prevalent in female athletes than non-athletes. Athletes participating in sport that emphasizes low body weight such as aesthetic and endurance athletes are particularly susceptible to menstrual abberations. There is great controversy as to whether athletic amenorrhea is due to a single entity related to exercise or whether it is mulifactorial. Menstrual irregularities associated with exercise include luteal phase deficiency, anovulation, and exercise-associated amenorrhea. Although the etiology of menstrual irregularities is unknown, there are several factors that predispose aesthetic athletes to such irregularities including, low body weight, weight loss, training intensity and the stress of physical training. These predisposing factors have led to hormonal responses responsible for reproductive alterations.

Aesthetic adolescent athletes are faced with energy concerns in an attempt to keep their body weight low. Low caloric intakes, low fat diets, eating disorders and restrained eating are associated with menstrual abnormalities in athletes and in the general population. A negative energy balance, as a result of inadequate energy relative to energy expenditure is implicated in the etiology of menstrual abnormalities. Additional health concerns of a negative energy balance include delayed growth and development in children and adolescents and a phenomenon called the "Female Athlete Triad". It is hypothesized that a negative energy balance results in reduction in resting energy expenditure and the thermic effect of food in an attempt to conserve energy for the body's vital functions. The following review of literature discusses the most current information regarding menstrual abnormalities of female athletes and the role of energy availability in menstrual function and energy conservation.

II. Menstrual Abnormalities Associated with Exercise

There are three main types of menstrual irregularities observed in athletes. They are, in sequence of increasing severity; luteal phase deficiency, anovulation and exercise associated amenorrhea. It should be noted that these menstrual cycle abnormalities are also common in adolescents who are not athletes.

1. Luteal Phase Deficiency

Luteal phase deficiency is characterized by insufficient progesterone production and the luteal phase may or may not be shortened to less than 10 days (Bonen et al., 1981 & Loucks, 1990). Luteal phase deficiency is also referred to as luteal phase inadequacy suggesting that the corpus luteum is dysfunctional with insufficient progesterone production as a result (Highet, 1989). Luteal phase deficiency is difficult to detect by researchers because total menstrual cycle length is usually normal and women do not usually notice any change (Otis, 1992). Because luteal phase deficiency goes undetected, the incidence of infertility among athletes is suggested to be much greater than reported (Keizer & Rogol, 1990). The reported risks of luteal phase deficiency include infertility, recurrent spontaneous abortions, endometrial hyperplasia, adenocarcinoma (Shangold, 1988) and decreased bone density (Prior, 1990). Frequent sampling throughout the menstrual cycle to detect the day of the LH peak and the progression of the progesterone concentration in the luteal phase is currently the accepted method for detecting luteal phase deficiency (Otis, 1992).

It has been well documented that extreme levels of exercise often result in luteal phase deficiency. Short luteal phase cycles and lowered progesterone levels despite an LH surge were first recognized in young swimmers when compared with non-exercising females of similar age (Bonen et al., 1981). The authors suggested this was a result of nonluteinization of the follicle. Shangold et al. (1979) reported a negative correlation between weekly running mileage and luteal phase length in distance runners. More recently, Bullen et al. (1985) showed that low progesterone levels and absent LH peaks could be induced by strenuous exercise in untrained women. Even mild exercise regimens can lead to suppressed luteal phase lengths and progesterone production (Ellison & Lager, 1986, Shangold et al., 1979).

Many researchers attribute the development of shortened luteal phase lengths to imbalances of LH and FSH, with inadequate follicular development (Strott et al., 1970, Sherman & Korenman 1974, Soules et al., 1984). By inducing early follicular decreases in FSH in rhesus monkeys, decreases in follicular phase estradiol and luteal phase progesterone production followed (Stouffer & Hodgen, 1980). These data suggest that a relative decrease in FSH may be responsible for the inadequate follicular development. However, the mechanism causing this decrease in FSH is unknown.

2. Anovulation

Anovulation is also referred to as anovulatory oligomenorrhea. Anovulation exhibits one of several different types of irregular bleeding patterns from very short cycles of less than 21 days to long cycles with 35 to 150 days between bleeding (Otis, 1992). The latter phenomenon is also referred to as oligomenorrhea. Estrogen is produced, however the basal body temperature (BBT) rise associated with ovulation is absent and low levels of progesterone result. Progesterone is required for endometrial sloughing, so the unopposed estrogen production leads to continuous endometrial stimulation and as a consequence, possible endometrial hyperplasia and adenocarcinoma. Bleeding is unpredictable and women usually suffer from menorrhagia, which can lead to iron depletion and anemia (Otis, 1992).

Studies have shown that even regularly menstruating athletes frequently show a shortened luteal phase and have anovulatory cycles. Prior et al. (1982) studied 24 females training for a marathon. Although all were regularly menstruating, only a third exhibited normal luteal phase lengths, a third were anovulatory and a third had a short luteal phase length. Loucks and Horvath, (1985) suggest anovulation is due to inappropriate steroid feedback to the hypothalamus or pituitary. Athletes with anovulation due to inappropriate feedback display an elevated LH pattern with LH concentrations greater than 25 mIU/ml and an inappropriate increase in the FSH/LH ratio during the follicular phase (Rebar, 1983). An inappropriate FSH/LH ratio (low FSH in comparison to LH levels) during the follicular phase is suggestive of an inadequate follicular development (Bonen et al., 1981).

3. Exercise-Associated Amenorrhea (EAA)

Exercise associated amenorrhea is also referred to as hypoestrogenic amenorrhea and is a form of hypothalamic amenorrhea. It is the most frequently observed menstrual abnormality in athletes (Otis, 1992). Amenorrhea is divided into two categories; primary amenorrhea and secondary amenorrhea. Primary amenorrhea is the delay of menarche beyond age 16. Menarche has been reported to be significantly delayed in aesthetic athletes in comparison to sedentary controls (Warren 1980, Malina 1983, Lindholm et al. 1994). Secondary amenorrhea in its most generalized definition is the absence of menstruation in women who previously were periodically menstrual (Loucks & Horvath, 1992). Secondary amenorrhea has been defined as the absence of 3 to 12 consecutive menstrual cycles. The observed prevalence is higher in athletes ranging from 3.4 to 66 percent, with the highest prevalence observed in distance runners (Loucks, 1985). The lack of universally accepted definitions of amenorrhea contribute to the conflicting results regarding the incidence of menstrual abnormalities and makes comparison of studies difficult (Loucks & Horvath, 1985).

Younger athletes who train intensely, may be at a greater risk for the development of athletic amenorrhea as a result of their immature hypothalamic-pituitary-ovarian axis compared to more mature athletes who train only moderately. Athletic amenorrhea also seems to be more prevalent among athletes who experienced menstrual irregularities prior to the onset of training (Malina, 1983).

III. Predisposing Factors Implicated in Menstrual Abnormalities in Athletes

A number of predisposing variables are hypothesized to contribute to the development of menstrual abnormalities in female athletes. These factors include low body fat, weight loss, training intensity, type of sport, and the mental stress of physical training and competition. Although a physiological mechanism responsible for the etiology of athletic amenorrhea is unknown at this time, it is suggested that it is a combination of many factors acting synergistically to interfere with the hypothalamic-pituitary-ovarian axis (Highet, 1989). An immature hypothalamic-pituitary-ovarian axis, characteristic of adolescence is also a predisposing factor to menstrual abnormalities.

1. Body Composition and Weight Loss

The frequency of leanness in athletes experiencing menstrual dysfunction is high (Bale et al., 1996). Oligomenorrheic and amenorrheic runners have been found to have lower relative fat content (Carlberg et al., 1983) and lower percent body fat than runners with normal menstrual cycles who were leaner than non-athlete women (Schwartz et al., 1981). Frisch and McArthur (1974) first proposed that amenorrhea was induced by changes in body composition. It was once thought that 17 percent body fat was required for the initiation of regular cycles and 22 percent body fat was a requisite for the maintenance of regular cycles (Frisch and McArthur, 1974). This theory has been discarded by many, as athletes with low body fat have been found to display normal menstrual cycles, while some athletes with a high percentage of body fat exhibit amenorrhea (Baker et al. 198, McArthur et al. 1980).

An alteration in body composition is hypothesized to alter the levels of estrogen and androgens and thus change feedback to the hypothalamus or pituitary since adipose tissue is one of the conversion sites for androgens and estrogens (Loucks 1990). Estradiol can be metabolized by either 16 α hydroxylation to form estriol (E_3) or via 2-hydroxylation forming the catecholestrogen 2-hydroxyestrone. The primary catabolite of estrogen in obese women is estriol, which is more estrogenic than 2-hydroxyestrone. which has low estrogenic activity and may even behave as an antiestrogen (Shoupe, 1991). Underweight women have been reported as having enhanced 2-hydroxyestrone production. Thus, body weight and adipose tissues stores influences estrogen metabolism by tending to make underweight women hypoestrogenic and overweight women hyperestrogenic (Shoupe, 1991).

The rate of weight or fat loss is suggested to play more of an important role in the etiology of amenorrhea as amenorrheic runners have reported losing more weight than eumenorrheic runners since the initiation of training (Ellison, 1981, Baker et al., 1981, Schwartz et al., 1981). Ninety-four percent of women subjected to an intense physical training regime combined with a weight loss diet reported menstrual dysfunction compared to 75 % of women who were randomized to a weight maintenance group combined with the same physical training program (Bullen et al., 1985).

2. Training Intensity and Sport Specificity

The duration and frequency of training has been considered in the etiology of athletic amenorrhea. Exercise is thought to alter hormone concentrations, resulting in changes in feedback to the hypothalamus or pituitary, so that the more an athlete trains, the longer the hormones may stay elevated and influence feedback. Periods of rest from exercise due to injury, have been reported to precipitate menarche and resumption of menses in the absence of any change in body weight in ballet dancers (Warren, 1980). A positive correlation between weekly running mileage and the incidence of athletic amenorrhea have been reported (Feicht et al., 1978, Lutter & Cushman 1982). Drinkwater et al. (1984) reported that amenorrheic runners ran an average of 27 km more per week than eumenorrheic runners did. However, there is still not a clear relationship between training mileage and menstrual status. In all surveys to date, there are still women who remain eumenorrheic with mileage equivalent to their amenorrheic counterparts. Bullen et al. (1985) suggests that the rate at which the intensity of training is increased may be more important in the onset of amenorrhea.

It is hypothesized that exercise associated amenorrhea may be sport specific, with the incidence being greatest in runners and ballet dancers compared to swimmers and cyclists (Sanborn et al., 1982). Loucks (1990) suggests this may be due to differences in training intensity or differences in body composition, or it may be that runners and dancers experience a greater elevation in core temperature than swimmers and cyclists who have the cool water and air to help regulate their core temperature. There also seems to be a relationship between athletes participating in sports, which emphasize leanness.

Retrospective and cross-sectional studies have found aesthetic athletes such as gymnasts and ballet dancers to have a later age of menarche compared to swimmers (Theintz et al., 1993), non-athlete controls (Lindholm et al., 1994, Warren, 1980) and non-aesthetic athletes (Malina, 1982). Gymnasts, figure skaters, divers and ballet dancers were found to have the latest mean menarcheal age in a comparative survey which examined 600 athletes in high school, university varsity level and Olympic level athletes with a greater percentage of late-maturing individuals at the more advanced levels of competition (Malina, 1982). In addition, Lindholm et al. (1994) found

menarche to be later in gymnasts compared with gymnasts' own mothers. The age at entry into sport training seems to play a role as Frisch et al. (1981) found that delayed menarche was dependent on whether training began before or after menarche. This may explain the high prevalence of late menarche in aesthetic athletes as gymnasts, dancers and figure skaters tend to start training at an early age (Malina 1982).

3. Mental Stress of Physical Training

The mental stress of physical exercise and competition is suggested to play a role in the disruption of normal reproduction function. The stress and tension of training and competition may increase the secretion of cortisol from the adrenal cortex, which disrupts the hypothalamic control of GnRH (Frisch et al., 1981). Warren (1980) evaluated the role stress played in the etiology of amenorrhea by studying dancers and young musicians who had similar goal-oriented lifestyles at an early age. The musicians reached menarche significantly earlier than the dancers, suggesting that physical stress is more important than psychological stress in delaying menarche. No difference in psychological stress has been reported in amenorrheic and eumenorrheic runners and non-athlete controls in measures of depression, hypochondriasis, obsessive compulsive tendencies, or recent stressful events. However, the amenorrheic runners scored significantly higher when subjectively ranking the stress of running on a scale of one to ten in comparison to the eumenorrheic runners (Schwartz et al., 1981).

IV. Hormonal Alterations Associated with Exercise

Physical training is associated with many of the changes that occur to the female reproductive physiology. The etiology of menstrual abnormalities is unclear because there are many factors implicated which are difficult to isolate because they coincide with athletic training. With exercise there are usually changes in body weight and body composition, eating behaviours, energy utilization and hormone levels (Highet, 1989). Individual factors such as hereditary, diet and stress may also play a role in menstrual irregularity.

Exercise may affect the production, metabolism, utilization, clearance of plasma hormones and plasma volume (Shangold, 1994). During continuous aerobic exercise

there is a transient increase in blood levels of several hormones. The long term effects of chronic repeated bouts of intense training are unknown. Acute alterations in response to exercise include increases in circulating concentrations of estradiol, progesterone, prolactin and testosterone, all of which return to normal within one to two hours following exercise. Exercise-induced alterations in one or more of the menstrual cycle hormones may disturb the normal menstrual cycle by disruption of feedback to the hypothalamus (Bonen et al., 1981).

1. Alterations in the Gonadotropins with Training

The concentration of LH has been reported as being depressed in the luteal phase with training (Jurkowski et al. 1978, Keizer, 1986). Boyden et al. (1984) have shown that LH levels in eumenorrheic women decrease with endurance training. LH has been reported to be significantly elevated in the follicular phase and FSH significantly depressed in the follicular phase, so that the FSH/LH ratio was far below normal in swimmers with short luteal phases in comparison to women with regular cycles (Bonen et al. 1981). Alterations in the gonadotropins may lead to inadequate follicle and corpus luteum development and thus a compromised estrogen and progesterone production.

Studies before the 1980's, which examine the resting levels of gonadotropins. suffer from technical and design flaws, using only single blood samples, which do not provide accurate estimates of resting hormones levels. With the refinement of recent sampling techniques, LH pulse frequency and amplitude have been measured, which is a more applicable and superior method of determining the characterization of hypothalamic function than mean gonadotropin concentration. Veldhuis et al. (1985) were the first to use this method of sampling in oligomenorrheic/amenorrheic runners and eumenorrheic non-athlete controls. LH pulse frequency was significantly lower in the amenorrheic athletes in comparison to the eumenorrheic controls, with LH secretory patterns similar to that of prepubertal females in some of the amenorrheic runners. Loucks et al. (1989) later confirmed these results as LH was reported to be lower in amenorrheic athletes in comparison to eumenorrheic athletes, who in turn have lower pulse frequencies in comparison to sedentary controls. Recently, Loucks et al., (1998)

reported that low energy availability not the stress of exercise disrupts LH pulsatility in exercising women. Williams et al. (1995) also found that only intense exercise in combination with diet restriction reduces LH pulse frequency with no change in mean LH levels and peak LH. However, it is important to note that some normally menstruating runners also have reduced LH secretion (Cumming et al., 1985 & Loucks et al., 1989).

Studies examining the effects of LH response to an exogenous bolus of GnRH report that amenorrheic athletes display an enhanced LH response compared to eumenorrheic athletes (Loucks et al., 1989 & Veldhuis et al., 1985). This evidence suggests that the decreased LH pulsatility reported in amenorrheic athletes is not a result of decreased pituitary responsiveness but rather inhibition of GnRH at the level of the hypothalamus or higher CNS centres that modulate hypothalamic activity.

2. Alterations in Ovarian Hormones with Training

Acute bouts of exercise for as little as 30 minutes at greater than 70 % of VO2 max have been reported to result in a significant increase in both estrogen and progesterone concentrations during the luteal phase (Bonen et al., 1979). Jurkowski et al., (1978) reported even greater progesterone concentrations in women exercising at 33 % of VO2 max to exhaustion. These increased concentrations of estrogen and progesterone could be a result of a reduced clearance rate, since exercise substantially reduces the hepatic blood flow (Wahren et al., 1971). The effects of acute exercise on the menstrual cycle is unknown. However if exercise is daily and prolonged, the elevated ovarian hormones could inhibit LH and FSH, compromising follicular and corpus luteum development and eventually suppress serum estrogen and progesterone levels (Bonen et al., 1981, Jurkowski et al., 1978).

The mechanism responsible for suppressed ovarian hormone levels is postulated to be a result of ineffective LH stimulation, specifically a reduced LH pulse frequency from the pituitary, which is hypothesized to be a result of ineffective GnRH at the level of the hypothalamus (Loucks & Horvath, 1985). Comparison of hormone profiles of the eumenorrheic athlete and the non-athlete adult suggest that luteal phase progesterone significantly decreases in athletes, as well as both luteal and follicular

phase estradiol levels (Shangold et al., 1979, Loucks, 1990). Previously sedentary women subjected to a two-month intense exercise training program developed menstrual irregularites, low luteal phase progesterone levels and diminished LH surges (Bullen et al., 1985). Ellison and Lager (1986) have shown that even moderate recreational activity such as running an average of 12.5 miles per week is associated with significantly reduced luteal phase progesterone levels. Reduced luteal phase progesterone levels have also been found to be associated with moderate weight loss due to dieting in the absence of exercise (Ellison & Lager, 1986).

2.1. Measurement of Salivary Progesterone

The average concentration of progesterone over the entire luteal phase can assess corpus luteum function. (Zorn et al., 1984, Read et al., 1985, Ellison, 1992). Salivary progesterone profiles determined over the luteal phase by radioimmunoassay have been reported to be more sensitive and less ambiguous indicator of corpus luteum function than basal body temperature recordings and more convenient than venous sampling (Ellison, 1993). Proper characterization of luteal function requires repeated samples at short intervals across the luteal phase. The noninvasive, convenient sampling technique and the stability of the samples are advantages to salivary sampling over venous sampling for the measurement of progesterone and thus corpus luteum function (Ellison, 1993).

The concentration of progesterone in saliva is independent of the salivary flow rate, reflects the total circulating concentrations in plasma and is considered to reflect the free fraction of the steroid (Ellison, 1993). Correlations obtained between plasma and salivary progesterone concentrations measured in the same subjects have been quite high (between 0.8 and 1.0) (Zorn et al., 1984, Ellison, 1993). Normal luteal phase salivary progesterone concentrations of the normal menstrual cycle range from 100 to 200 pg/ml (Choe et al., 1983, Zorn et al., 1984, Vuorento et al., 1989). Luteal phase progesterone concentrations are much lower in luteal insufficiency. It is difficult to diagnose the level of inadequate corpus luteum function that would impair fertility, because this requires serial hormonal measurement and endometrial biopsies (McNeely & Soules, 1988). By plotting the cumulative production of salivary progesterone across

the luteal phase against the cumulative time elapsed, normal and abnormal luteal function can be assessed (Ellison, 1993).

3. Alterations in Cortisol with Training

Physical training is hypothesized to affect the menstrual cycle by its involvement in stress and activation of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis has been demonstrated to down regulate the HPO axis in both animals and humans (Rivier & Rivest, 1991). Amenorrheic runners are reported to have hypercortisolism (Ding et al., 1988) with resting serum cortisol concentrations reported to be higher in both amenorrheic and eumenorrheic athletes compared to eumenorrheic sedentary controls (Loucks et al., 1989, DeSouza & Metzger, 1991). Patients with anorexia nervosa also present with hypercortisolism although it is more severe than the hypercortisolism that occurs with athletic amenorrhea, suggesting a different etiology for the two types of amenorrhea (DeSouza &Metzger, 1991).

The secretion of cortisol is regulated by a negative feedback system involving the hypothalamic-pituitary-adrenal axis. The hypothalamus releases corticotropin-releasing hormone (CRH) which stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH in turn stimulates the adrenal cortex to synthesize and secrete cortisol. Thus cortisol secretion is regulated by ACTH through a negative feedback system. Animal research has shown that CRH may be implicated in the development of menstrual abnormalities as CRH can directly inhibit GnRH release from the hypothalamus. (Gambacciani et al., 1986). It is also postulated that CRH may act with beta-endorphin since ACTH and beta-endorphin are derived from the same precursor, pro-opiomelanocortin (Loucks, 1990). Despite hypercortisolism, little difference has been reported in ACTH secretion in eumenorrheic runners and eumenorrheic sedentary women (Loucks et al., 1989). Exogenous administered CRH resulted in reduced sensitivity of the pituitary in both amenorrheic and eumenorrheic compared to eumenorrheic sedentary controls (Loucks et al, 1989). Therefore, the authors concluded that the normal ACTH levels despite CRH stimulation and hypercortisolism is suggested to be a result of loss of negative feedback of cortisol at the level of the hypothalamus, leading to increased CRH levels in the pituitary.

4. Antireproductive Hormones

Chronic physical activity increases the concentration of endogenous opioids (Mack et al., 1990) and prolactin (Samuels et al., 1991). Endogenous opioids, and prolactin are classified as antireproductive hormones because of their effects on the hypothalamic-pituitary axis. Endogenous opioids play a role in pain sensation, thermoregulation, appetite and reproduction and are hypothesized to play a role in menstrual dysfunction. They are widely distributed throughout the body with the highest concentration in the hypothalamus (Ferin & Vande Wiele, 1984). Amenorrheic athletes are reported as having greater resting endorphin levels than eumenorrheic sedentary controls (Laatikainen et al., 1986). This finding is not surprising since exercise has been found to yield a 2 to 5 fold increase in endorphins (Bortz et al., 1981, Viswanathan et al., 1987), and the intensity of training increases secretion of beta-endorphin (Russel et al., 1984).

Endorphins administered to both animals (Van Vugt et al., 1983, Ferrin et al.. 1982) and humans (Reid et al., 1981) result in an inhibition of LH secretion. Endogenous opioids are hypothesized to control and suppress gonadotropin secretion by modulating the oscillator in the arcuate nucleus, which is responsible for regulating the pulse frequency of the gonadotrophs (Ferin & Vande Wiele 1984). Studies using naloxone, an opiate receptor antagonist have shown that naloxone destroys the inhibitory effects of opioids on gonadotropins and induces an increase in LH in both animals and humans (Quigly and Yen 1980, Van Vugt et al., 1983). However, some researchers have found no response in amenorrheic athletes after administration of naloxone (Cumming et al., 1985, Samuels et al., 1991). Harber et al. (1997) found that both amenorrheic and eumenorrheic athletes have higher beta-endorphin levels than eumenorrheic sedentary controls, with no difference between amenorrheic and eumenorrheic athletes. The authors concluded that the higher beta-endorphin levels in the athletes were related to intense training, and not athletic amenorrhea.

Resting prolactin levels are reported as being decreased in female athletes, whereas intense exercise in trained athletes' results in increases up to 1000-fold immediately after exercise. Prolactin is a polypeptide hormone synthesized and secreted from the anterior pituitary. Although prolactin does not appear to play a

physiological role in the regulation of gonadal function, hyperprolactinemia in humans leads to hypogonadism. Hyperprolactinemia has been implicated in the etiology of athletic amenorrhea as elevated prolactin interferes with GnRH function (Marthur et al., 1986). Conflicting results exist, with no difference being reported between runners and sedentary controls over repeated sampling (Chang et al., 1984). Opioids and prolactin may act synergistically to inhibit GnRH as opioids play a role in increasing prolactin levels by the suppression of dopamine secretion. Thus the post-exercise increase in prolactin may be initiated by endogenous opioid release (Highet 1989).

V. Nutritional Factors Associated with Menstrual Abnormalities in Athletes

1. Low Energy Intakes in Athletes

Endocrine dysfunction is common among chronically undernourished individuals (Warren, 1983). Dieting is a common practice among athletes, especially aesthetic athletes where low body weight and a low percentage of body fat may provide a competitive advantage. The data presented in Table 2.1 are a summary of studies investigating energy intakes of aesthetic athletes. The mean caloric intake of the athletes in the studies summarized ranged from 1174 to 1930 kcalories/day which is well below both the Canadian Recommended Nutrient Intakes (RNI) and the United States Recommended Daily Allowances (RDA). The current Canadian RNI suggests that females 13-15 years of age consume 2200 kcal/day; 16-24 years, 2100 kcal/day; 25-49 years, 1900 kcal/day. The US RDA suggests that females 11-24 years of age consume 2200 kcal/day. These energy requirements are for a healthy population of median weights and generalized activity levels of sedentary to light. The athletes in the studies would have greater energy requirements than the average population since their activity levels are much higher than the average population. This puts the athletes even further below the average recommendations for energy intake and possibly at a negative energy balance. However, because actual energy requirements of young athletes have not been determined, it is difficult to conclude whether the energy needs of the athletes were met.

The few studies that used non-athlete controls found that some controls were also consuming less than the recommendation for energy (Kirchner et al., 1995), and that when energy intake was expressed as a kcal/kg ratio, there was no difference between athletes and non-athletes (Benson et al., 1990). Although many of the studies did not correct for body weight it is important to consider as it is possible that aesthetic athletes who are typically smaller than the average population are not restricting calories but that their smaller body size may influence their energy intake (Kirchner et al., 1995). When interpreting and comparing the results of these studies, the use of appropriate controls, sample size and method of energy intake collection must be considered. The accuracy of determining energy intake of individuals from studies using self-report data to assess energy intake has limited accuracy as young people, like adults have been found to under-report energy intakes (Edwards, 1993) and young populations may be prone to recording errors (Thompson, 1998).

Table 2.1. Summary of Studies Investigating Energy Intakes in Aesthetic Female Athletes

Study	Sport	Controls	Mean Age (years)	Mean Wt (kg)	Energy Intake (kcal)	Energy Intake (kcal/kg)	Dietary Recall Method
Benson et al. (1990)	Gymnast n = 12 Swimmers n = 18	12	12.5 ± 1.1 12.8 ± 0.9	34.9 ± 6.0 47.9 ± 8.9	1544 ± 398 1892 ± 446	39.4 ± 13.3 39.5 ± 10.6	1, 7-day food record
Bernadot et al. (1989)	Gymnasts n = 51	X	9.4	30.6	1651 ± 363	NA	2, 24-hour recalls
Calabrese et al. (1983)	Ballet Dancers n = 25	X	21.9 ± 4.3	53.1 ± 6.6	1358	NA	1, 3-day food record
Calabrese et al. (1985)	Gymnasts n = 97	X	14.8	43.5	1744	NA	1, 3-day food record
Ersoy (1991)	Gymnasts n = 20	20	11.5 ± 0.5	31.6 ± 1.5	1568	NA	1, 3-day food record
Hickson et al. (1986)	Gymnasts n = 9 Basketball n = 13	X	19.1 ± 0.6 19.4 ± 0.3	58.0 ± 3.0 68.3 ± 1.6	1827 ± 182 1932 ± 116	32 ± 12 30 ± 8	1, 24-hour recall
Kirchener et al. (1995)	Gymnasts n = 26	26	19.7 ± 0.2	54.1 ± 1.2	1381 ± 109	NA	FFQ and 1. 4-day food record
Lindholm et al. (1995)	Gymnasts n = 22	22	14.8	46.8	1930 ± 455	NA	2, 7-day weighed food records
Loosli et al. (1986)	Gymnasts n = 97	X	13.1	43.2	1838		1, 3-day food record
Moffat (1984)	Gymnasts n = 13	X	15.2 ± 4.1	50.4 ± 6.5	1923 ± 674	NA	2, 3-day food records
Reggiani et al. (1989)	Gymnasts N = 26	X	12.3 ± 1.7	37.9 ± 6.9	1552 ± 509	42.8 ± 17.6	1 weekly weighed food record
Rucinski (1989)	Figure Skaters n = 23	X	13 - 22 (range)		1174		
van Erp-Baart et al. (1985)	Gymnasts n = 11	X	15.4 ± 1.4	48.0 ± 8	1510		1, 7-day food record

2. Eating Disorders

In the last decade the incidence of eating disorders in athletes has increased (Sundgot-Borgen, 1994). Clinical eating disorders include anorexia nervosa (AN), bulimia nervosa (BN), binge eating disorder (BED) and eating disorder not otherwise

specified (EDNOS). There are strict psychiatric criteria that must be satisfied in order to make a conclusive diagnosis of a clinical eating disorder. However there is a spectrum of disordered eating patterns that ranges from a preoccupation with food and body image to frank anorexia nervosa or bulimia nervosa. Subclinical eating disorders are a form of eating disorders that do not meet the strict criteria for AN or BN. Anorexia athletica is a form of subclinical eating disorder that has been recognized among athletes, where the female athlete demonstrates an intense fear of gaining weight even though she is underweight (at least 5% less than expected for age and height for the general female population) (Sundgot-Borgen, 1993). Low body weight is accomplished by restricting energy intake and/or excessive exercise. The athlete with anorexia athletica will also report bingeing, self-induced vomiting, the use of laxatives or diuretics or all three.

Athletes are thought to possess characteristics associated with individuals who are at high risk of developing eating disorders (Clifton 1991, Wilmore, 1992). High self-expectation, perfectionism, persistence and independence are characteristics thought to be risk factors in the development of eating disorders (Sundgot-Borgen, 1996). These characteristic traits may enable individuals to succeed in sport but may also place athletes at an increased risk of developing an eating disorder. Athletes involved in aesthetic sports that emphasize leanness are reported to be at the highest risk of developing an eating disorder (Sundgot-Borgen, 1993, Sundgot-Borgen & Corben, 1987, Rosen, 1988). Aesthetic athletes have been found to have similar attitudes and behaviours towards maintaining a low body weight such as food restriction, excessive exercise and a concern for a slim physique, as patients with anorexia nervosa (Bale et al., 1996).

Figure skaters (Rucinski, 1989) and ballet dancers are reported to have a high prevalence of eating disorders or eating disorder tendencies (Brooks-Gunn et al, 1987, Evers, 1987, Hamilton et al. 1985). Abraham (1996) reported that 50 percent of dancers had anorexia or bulimia at some time, and that 15 percent of elite ballet dancers were found to have clinical AN and an additional 50 % exhibited anorectic tendencies. Other studies have failed to show that aesthetic athletes are at a greater risk of developing an eating disorder, with gymnasts exhibiting fewer tendencies towards eating disorders

than swimmers (O'Connor et al., 1996, Benson et al., 1990). However, in another study, gymnasts reported using more pathogenic weight control techniques and having more weight concerns and a greater preoccupation with thinness, than non-athlete controls (O'Connor et al., 1995).

An individual's body type is hypothesized to influence whether an athlete will be successful (Brownell et al., 1987). Training for a specific sport before the body matures might prohibit athletes from choosing a suitable sport for their adult body type and may elicit conflict when athletes struggle to prevent the natural physical changes that accompany puberty (Sundgot-Borgen, 1994). Early participation in sport specific training has been associated with eating disorders in gymnasts (Rosen and Hough, 1988). An explanation for the lower than expected incidence of eating disorders reported among gymnasts may be that they selected their sport because they already had lean and small bodies. In addition, coaches may have encouraged girls with naturally thin bodies to continue on to the elite level of competition (Benson et al., 1990, O'Connor et al., 1996).

Depending on the method of assessment used, the prevalence of eating disorders among female athletes has been reported to be as low as 1% to as high as 39.2% (Sundgot-Borgen, 1993). Precise estimates of prevalence are difficult because of the secretive nature of the disorders and the diverse definitions of the disorders among studies. Most studies have used self-report questionnaires such as the Eating Disorder Inventory (EDI) (Garner et al., 1984) and the Eating Attitude Test (EAT) (Garner & Garfunkel, 1979) despite their questionable validity. The validity of the EAT has not been tested in athletes and when the EDI was given to athletes, it was found to be unreliable in predicting actual eating disorder incidence (Wilmore et al., 1992). However, Sundgot-Borgen, (1993) found that the EDI accurately predicted prevalence of eating disorders in 89 percent of the athletes when interviewed.

3. Macronutrient Content of the Diet

Although only speculated at present, diet or nutritional status may also play a role in menstrual irregularities. Besides overall energy intake, protein, vitamin A, fibre, and fat intake have been considered in the etiology of athletic amenorrhea. Diets

deficient in certain amino acids have been shown to alter neurotransmitter synthesis, which is speculated to affect the secretion of GnRH (Loucks & Horvath, 1985). Studies have cited that amenorrheic runners consume significantly less protein than eumenorrheic runners (Marcus et al. 1985, Cohen et al., 1985, Hamilton et al., 1986, Drinkwater et al.,1984, Bruemmer at al., 1987). However, because the amenorrheic runners also consumed significantly less total calories, the relative amount of calories from protein in the majority of the studies was not different. Body composition and training intensity was also quite different between the two groups (Drinkwater et al.,1984, Bruemmer at al., 1987).

Total fat intake and the percentage of calories from fat has been reported to be significantly lower in the diets of amenorrheic athletes in comparison to eumenorrheic athletes (Deuster et al., 1986, Brooks et al., 1984, Pederson et al., 1991, Lloyd et al., 1987). A positive correlation between dietary fat intake and plasma estrogen levels has been reported (Goldin et al., Unpublished data from Aldercreutz et al., 1986). A high fat diet is suggested to lead to an elevated fat concentration in the intestine, which may increase the reabsorption of lipid soluble estrogens (Aldercreutz et al., 1986). These findings suggest that the amount of fat consumed may be involved in regulating estrogen production and excretion.

Vitamin A intakes, primarily in the form of beta-carotene and other carotenoids have been reported as being excessively high in the diets of amenorrheic athletes (Deuster et al., 1986, Marcus et al., 1985). Also, diets high in beta-carotene have been associated with reproductive dysfunction in animals. Sherwood et al. (1937) noted changes in the estrous cycle of the rat, and Gellert, (1977) reported inhibited ovulatory activity in the rat fed high doses of vitamin A. In humans, hypothalamic amenorrhea has been described in association with hypercarotenemia in women (Kemmann et al., 1983). Beta-carotene, unlike vitamin A is deposited in adipose tissue and the corpus luteum (Wolf, 1984). A clinical condition termed "golden ovaries" is characterized by excessive intakes of beta-carotene, elevated carotene in the ovaries and menstrual disturbances (Page, 1971). Although it is unlikely that excessive beta-carotene intakes are directly related to menstrual irregularities, excessive intakes may act indirectly to induce metabolic changes that lead to menstrual disturbances (Deuster et al., 1986).

4. Vegetarianism

Vegetarianism is prevalent in female athletes (Slavin et al., 1984, Brooks et al., 1984), and the incidence in amenorrheic athletes appears to be greater than it is in eumenorrheic athletes (Bruemmer and Drinkwater 1987, Slavin et al., 1984, Pederson et al., 1991). The mechanism as to how vegetarian diets influence the menstrual cycle is not clear, although the macronutrient content of these diets appears to be related to estrogen metabolism. Vegetarian diets are high in fibre, low in fat, and particularly low in saturated fat. Oligomenorrheic athletes have been found to consume significantly more dietary fibre than eumenorrheic athletes and eumenorrheic sedentary controls (Lloyd et al., 1987, Snow et al., 1990). Results of an intervention study showed that non-athlete women assigned to a low calorie vegetarian diet for 6 weeks developed significantly more menstrual irregularities, lower estrogen, progesterone, and LH hormone concentrations compared to women assigned to a non-vegetarian diet (Pirke et al., 1986). Conversely, Barr et al. (1994) reported that women consuming a vegetarian diet had fewer menstrual disturbances than non-vegetarian women. However, the vegetarian women had lower BMI's, percent body fat and dietary restraint scores than the non-vegetarian women. Therefore the lower incidence of menstrual irregularities among this group of women could be related to lower occurrences of eating problems and less of a preoccupation with food and dieting.

A low fat, high fibre diet has been reported to modify estrogen metabolism. When Oriental and Caucasian women were compared, a positive relationship between dietary fat intake and serum estradiol concentrations were found as well as a negative relationship between dietary fibre intake and serum estradiol concentrations (Goldin et al., 1986). The greater fecal excretion of estrogens and lower serum estrone and estradiol levels reported in vegetarian women compared to omnivorous women (Goldin et al., 1982, Woods et al., 1984, Schultz and Leklem, 1983) support this theory. A low fat, high fibre diet is thought to reduce the enterohepatic circulation of estrogens by altering the bacterial flora resulting in an altered intestinal absorption. Specifically, the ability of the intestinal flora to hydrolyse and conjugate biliary estrogen with B-glucuronidase is reduced and consequently so is the reabsorption of estrogens (Aldercreutz et al., 1986). Reduced B-glucuronidase activity has also been found in the

fecal bacteria of vegetarian women (Goldin et al., 1986, Aldercreutz, 1980). In addition, increased fibre consumption results in increased fecal bulk, a faster transit time and consequently a greater excretion of estrogen (Aldercreutz, 1980).

5. Undernutrition

Undernutrition plays an important role in reproductive function (Loucks & Horvath, 1985, Van Der Spuy, 1985). With improved lifestyles and nutrition status, the demographic data suggest that the age of menarche has decreased in the Western hemisphere (Tanner, 1973). Research on anorexia nervosa has led to a better understanding of the hypothalamic mechanisms affecting menstrual cycle disorders and delayed menarche since amenorrhea is an early symptom of AN. Athletic amenorrhea and amenorrhea associated with AN share many of the same characteristics. including weight loss, a high volume of physical activity, emotional and physical stress and they are both characterized by decreased levels of gonadotropins and estrogen (DeSouza & Metzger, 1991). However, differences in the physiological response to stress between these two types of amenorrhea exist. These differences are as follows; patients with AN appear to have an altered thyroid metabolism as shown by a shift from T4 to reverse T3 (Warren & Vande Wiele, 1973), which has been reported in athletes but not to the same extent (DeSouza & Metzger, 1991). The hypercortisolism which is present in both athletes and AN patients differs in magnitude; AN patients exhibit significantly greater cortisol levels than athletes (De Souza et al., 1991 & Ding et al., 1988). Gonadotropin pulsatility in athletes tends to be diminished with a reduced pulse frequency, whereas patients with AN tend to display a prepubertal pattern of gonadotropin pulses that occur nocturnally only (Nillius & Wide, 1977).

Starvation causes a pre-pubertal LH secretion pattern that is seen in AN patients and in normal girls before they reach puberty. Pirke et al. (1983) have shown that at least 59 % of ideal body weight (IBW) must be reached during recovery from AN before LH patterns develop again. Adult patterns of LH secretion are not reached until greater than 80 % of IBW is reached and menstrual cycles do not develop until 87 % of IBW is reached. These observations indicate gonadotropin secretion and menstrual cycles are weight dependent. However, about 50 % of women with bulimia nervosa are

amenorrheic or oligomenorrheic, despite normal body weight (Pirke et al.,1987). Similarly, Marimoto et al. (1980) reported that amenorrhea occurs with very little or no weight loss in half of AN patients, and the resumption of menstrual cycles does not always occur with a significant weight gain during recovery (Pirke et al., 1983, Walkeling et al., 1977). These data suggest that body weight is not solely responsible for menstrual dysfunction.

Patients with AN display abnormal secretion of gonadotropins which is hypothesized to be a result of inadequate GnRH secretion at the level of the hypothalamus (DeSouza & Metzger, 1991). Researchers have tested this hypothesis by administering a GnRH bolus and observing the responsiveness of the pituitary gland and the hypothalamus. GnRH administered to patients with severe AN resulted in a prepuburtal pattern of gonadotropin response. Specifically, FSH secretion is moderately increased and the LH response is absent (Warren & Vande Wiele, 1973, Nillius et al., 1975). This pattern is analogous to the pattern observed in normal prepubertal females with the administration of GnRH (Franchimont et al., 1974). Others have reported that patients with AN display a normal pituitary response of GnRH when administered in a pulsatile manner, with the gonadotropins returning to normal adult patterns 4 days after the administration (Marshall & Kelch, 1979). Women with athletic amenorrhea display an increased LH response to the administration of GnRH, suggesting that there are differences in the etiology of the two types of amenorrhea.

In order to learn more about the role undernutrition plays on reproductive dysfunction, it must be studied in isolation in the absence of AN, abnormal weight and excessive exercise. Administration of a low calorie diet for approximately two weeks to healthy young women of normal body weight resulted in regression of LH secretion to a pre-pubertal pattern and anovulation and shortened luteal phases in women who were previously ovulatory (Pirke et al., 1985). Loucks et al. (1994) reported that eumenorrheic, sedentary, normal weight women on a 4 day calorie restriction diet (10 kcal/kg/day) displayed a reduction of 23% in LH pulse frequency especially during the waking hours, and a 40% increased pulse frequency during sleep of the follicular phase, with no changes in mean LH, FSH and cortisol concentration. Women on the restricted

diet lost 1.5 kg body weight and were reported as being in negative energy balance. The authors concluded that LH pulsatility depends upon energy availability.

Acute periods of starvation for as little as 72 hours in normal weight women has also resulted in alterations in gonadotropin secretory patterns in the midfollicular phase (Olson et al., 1995). Specifically, the fasted subjects cycles displayed 20 % fewer number of LH pulses on the last day of the fast with no increase over time of mean LH values, LH area under the curve and LH pulse amplitude in comparison to the fed cycle. However, the cycles of subjects who were fasted and fed displayed similar luteal phase lengths and ovulation. The authors concluded that although a 72 hour fast is not long enough to cause altered follicular development and cycle lengths in sedentary normal weight women, a reduction in the number of LH pulses over 24 hours is evident. Alvero et al. (1998) performed a similar study, with lean sedentary women to determine whether the effects of a 72-hour fast would be more pronounced in lean women (< 20% body fat, BMI < 20, regular menstrual cycles, no psychiatric histories). Results of this study show striking similarities to those of Olson et al. (1995), with a 19 % decrease in the number of LH pulses over 24 hours and no differences in mean LH, LH amplitude and area under the curve and mean FSH levels. Anovulation and shortened luteal phases occurred in half of the women on the fasted cycles. The authors suggested that the ability of some of the women to maintain ovulation despite a high amount of lean mass compared to fat mass may be related to site-specific fat distribution. Although all women were less than 20 % body fat, body composition assessed by dual energy x-ray absorptiometry (DEXA) showed that these women were lean in the trunk compared to periphery.

6 Dietary Restraint

Dietary restraint plays a major role in weight regulation, weight maintenance, obesity and eating disorders (Allison, 1995). Restrained eating and weight reducing diets in the general population have been reported to affect the menstrual cycle (Barr et al., 1994, Schweiger et al., 1992), particularly follicular development and progesterone secretion during the luteal phase (Kurzer & Calloway, 1986 and Schweiger et al., 1987). Restrained eating is defined as a person's tendency to eat less than desired (Ruderman

1985). Restrained eaters do not share the same psychopathology as individuals suffering from an eating disorder although everyday eating is characterized by continuous dieting (Gorman & Allision, 1995). Mean energy intake has been reported to be more than 20% lower and usual weight tends to be slightly higher in restrained eaters in comparison to non-restrained eaters (Allison, 1995). 1989). Recently, Schweiger et al. (1992) examined the hypothesis that restrained eating is associated with an increase in the likelihood of menstrual disturbance. Women with low dietary restraint, were reported as having normal cycles and normal estradiol and progesterone concentrations, while the majority of the women with high restraint had reduced progesterone and shortened luteal phases. The authors conclude that high dietary restraint may be a risk factor for the development of menstrual disturbance in young women.

6.1 Measurement of Dietary Restraint

Restrained eating is assessed primarily by three questionnaires. These questionnaires include the Restraint Scale (RS) (Herman & Polivy,1980), the Eating Inventory (Stunkard & Messck, 1985), and the Dutch Eating Behaviour Questionnaire (DEBQR) (van Strein, et al., 1986). The Eating Inventory was adapted from an original questionnaire, the Three-Factor Eating Questionnaire (TFEQ) to assess other aspects of eating behaviour. Factor analysis of the TFEQ brought about three other aspects of eating behaviour; cognitive control of eating, disinhibition and susceptibility to hunger. These three aspects of eating behaviour were included in the Eating Inventory. Allison et al. (1992) performed factor analysis to identify overlap among the three scales and found that each scale measures different components of the restrained construct. For example, the TFEQ by Stunkard and Messick is the only scale to quantify behavioural restraint and the RS is the only questionnaire to take into account weight fluctuation.

6.1.i. The Dutch Eating Behaviour Questionnaire (DEBQR)

The DEBQR was developed because the other scales did not make a clear distinction among the constructs of eating behaviour such as restrained eating, emotionally triggered eating and eating in response to external or internal hunger cues.

Thus the DEBQR is cited as the best scale to assess dietary restraint (Gorman & Allison, 1995). The DEBQR initially consisted of 33 questions to assess restrained eating, emotional eating and external eating in a Likert scale. The restraint scale consists of 10 items which were derived through repeated factor and item analysis, and have been found to have an internal consistency of $\geq .90$ as measured by Cronbach's α (Gorman & Allison, 1995). A test-retest reliability of .92 has been reported over a 2 week period by Allison et al. (1992). The DEBQR is appropriate for adolescents as it has a reading level appropriate for 5th graders (Allison & Franklin, 1993). The DEBQR, however, has been reported as confounding two aspects of caloric restriction, those being intentions to diet and actual dieting success (Odgen, 1993).

VI. Role of Energy Conservation in Menstrual Function

1. Energy Balance

The energy requirement of humans is defined by the World Health Organization (WHO) as the level of energy intake from food which will balance energy expenditure when the individual has a body size and composition and level of physical activity consistent with long-term good health and growth (Wong, 1994). Energy balance occurs when energy requirements are met and is defined as the state when energy intake is equal to energy expenditure (Thompson, 1998). An energy imbalance results when energy intake does not match energy expenditure. A positive energy balance occurs when energy intake is greater than energy expenditure and is the state required for weight gain, growth and development (Thompson, 1998). A negative energy balance occurs when energy intake is less than energy expenditure, and usually results in weight loss.

The determination of energy balance requires the assessment of energy intake and energy expenditure. Energy intake is derived solely from the consumption of food and beverage. Total energy expenditure includes the energy expended for basal metabolism, thermic effect of food and for physical activity. Athletes are constantly manipulating the energy balance equation. By decreasing energy intake and increasing

energy expenditure a negative energy balance can be obtained which is often the favoured state of aesthetic athletes (Thompson 1998).

1.1 Measurement of Energy Intake

There are several methods to measure energy intake and the method of choice is determined by the specific circumstances of the investigation such as the subjects being studied and the aim of the study (Wolper, Heshka, Heymsfield, 1995). Food intake analysis can be classified by quantitative and qualitative methods (Gibson 1990). Quantitative dietary assessment methods consist of recalls or food records designed to measure the quantity of foods consumed over a set number of days. By increasing the number of measurement days, quantitative estimates of habitual food intake can be obtained. Quantitative dietary assessment methods include the 24-hour recall and the three-day food record. Qualitative dietary assessment methods have the ability to obtain retrospective information on the pattern of food use during a longer, less defined period of time (Gibson, 1990) and include the food frequency questionnaire and the dietary history. Qualitative methods are most frequently used to assess habitual intake of foods or specific classes of foods. Quantitative methods such as food records are recommended for the determination of energy intake.

1.1.i. Food Records

Food records are used to estimate intakes of individuals over a specified time period. Respondents record all food and beverage consumed for a specified period of time in an organized fashion. Food portions are estimated by using measuring cups, spoons, counts and rulers. Weighed food records require that all ingredients as well as inedible waste, the cooked weight of an individual portion and the table waste be weighed by a household food scale. This method is not frequently used in studies of free-living individuals as it demands a high degree of subject motivation and cannot be used when meals are consumed away from home. An advantage of food records is that they do not rely upon memory for accuracy and they have been found to provide an adequate estimation of energy intake during a designated time period (Wolper, Heshka, Heymsfield, 1995).

The number of days in which records are kept varies from three, five or seven days and is dependent upon the aim of the investigation, the subjects and the degree of accuracy of the estimate of usual intake (Gibson, 1990). Seven day food records are reported as being more accurate than the three or five day food records (Wolper, Heshka, Heymsfield, 1995). However, the greater the number of recording days, the higher the respondent burden and the lower the cooperation (Gibson, 1990). Doubly-labelled water has been used to validate seven-day food records (Schoeller, 1990) with poor validity as 20% fewer calories were reported than were actually consumed. Three days of food intake records have been found to be adequate in estimating group (n = 16) energy intakes with 95 percent confidence in females (Basiotis et al., 1987). In addition, the three-day food record requires less motivation of the participant and may limit the amount of intentional and unintentional recording. For example, in order to simplify the recording process, a subject might choose a sandwich instead of the salad bar because the former would be easier to record (Barr, 1987). Thus three day food records may be more appropriate for children and adolescents (Gibson, 1990). Errors can be reduced by providing precise instructions to subjects prior to data collection.

1.2. Measurement of Energy Expenditure

1.2.i. Measurement of Resting Energy Expenditure

REE is defined as the sum of the minimal activity of all tissue cells of the body under steady-state conditions, and it is frequently expressed as the rate of heat production or oxygen consumption related to some unit of body size (Schofield, 1995). REE accounts for 50-70 % of total energy expenditure in humans (Wong et al., 1996) and shows very little day to day variation (Garrel, 1996). REE is often used interchangeably with resting metabolic rate (RMR), differing only in units of expression. REE is the energy expended per day and RMR is the energy expended per minute or hour. REE is measured when the subject is fasted (12 hours) and after resting in a supine position for 30 minutes in a thermally neutral environment (Wong et al., 1996).

REE can be determined by indirect and direct calorimetry. The gold standard for measuring energy expenditure is by direct calorimetry as energy expended by the

body is ultimately released as heat. Despite high accuracy, the use of direct calorimetry is limited as the chambers are expensive to construct and operate. Indirect calorimetry involves measuring oxygen uptake, carbon dioxide expiration and urinary excretion of nitrogen. Heat production in the form of adenosine triphosphate (ATP), is derived primarily from substrate oxidation, and since the oxygen stores of the body are insignificant, the volume of oxygen consumed is closely correlated with the rate of heat production or energy expenditure (Schultz & Deurenberg, 1996). Indirect calorimetry assumes that all substrates are completely oxidized to CO_2 and H_2O and that urea is the primary end product of protein metabolism.

Estimation of the rate of substrate oxidation is also possible with indirect calorimetry. The quantity of CO_2 produced in relation to oxygen consumed varies depending on the substrate oxidized. The respiratory quotient (RQ) (defined as VCO_2 / VO_2) is the ratio of metabolic gas exchange in the oxidation of substrates under steady state conditions. A steady state exists when the O_2 uptake equals the O_2 requirement of the tissues and there is no accumulation of lactic acid and heart rate, ventilation and cardiac output remain at fairly constant levels (McArdle, Katch, Katch, 1996). With indirect calorimetry however, only an estimate of the net disappearance rate of substrates can be made, since intermediate metabolic processes cannot be assessed. An example would be when a substrate is converted into another one prior to its oxidation or utilized for other purposes (Schultz & Deurenberg, 1996).

REE can also be predicted from equations developed from large studies of indirect calorimetry measurements. Wong et al. (1996) evaluated 10 common REE prediction equations in female children and adolescents, which were validated against whole-body calorimetry. The equations proposed by WHO/FAO/UNO (WHO, 1985) and Schofield (1995) using both body weight and height in the calculation yielded the most accurate mean REE compared to the mean REE determined by whole body calorimetry (Wong et al., 1996).

1.2.i.a. Factors Affecting REE

There are a number of factors that affect REE. In general, REE is greater in individuals with greater lean body mass (LBM) (growing children, pregnant women and

males), greater body surface area (tall for weight) as well as those suffering from various illnesses, stress or hyperthyroidism (Whitney, Hamilton, Rolfes, 1990). REE is lowered in individuals with a decreased lean tissue, hypothyroidism, malnutrition or those who are fasting or inactive. With underfeeding there is a decrease in REE, which is the result of loss of tissue, and metabolic activity of the remaining tissue becomes depressed. Starvation and semi-starvation have been shown to depress metabolic rate between 15 to 30 % (Bray, 1969). Dieting and low caloric intakes are also associated with a decrease in REE. Reductions in REE from 9.5 % (Welle et al., 1984) to 15 % have been reported in obese women on a 472 kcal/day and 450kcal/day diet respectively (Bray, 1969). Restrained eaters have also been reported to have a lower resting metabolic rate (Poehlman et al., 1991, Bennett et al., 1989, Devlin et al., 1990). Over-feeding studies, are associated with an increase in REE, as greater metabolic energy is needed to maintain a gain in tissue mass (Mole 1990). Over-feeding non-obese women by 3000 kcal per day resulted in an elevated REE, while semi-starvation (about 500 kcal/day) for 2 weeks, resulted in a reduced REE.

REE and LBM are positively correlated (Webb 1981, Poehlman et al., 1998). It is suggested that the reduction in REE on low calorie diets may be prevented by the addition of exercise (Mole, 1990). Recent studies comparing the effects of very low calorie diets (VLCD) and VLCD's with the addition of aerobic and resistance training have shown conflicting results. Since resistance training is muscle building activity it is thought to preserve LBM on VLCD's. However, when VLCD's were examined alone and compared with VLCD's combined with resistance training, losses in LBM and REE were similar to those found on a VLCD alone (Donnelly et al.,1991, 1993, Lemons et al., 1989, Gornall & Villani, 1996).

Some evidence exists to suggest that endurance training and VO_2 max are related to an enhanced REE. Poehlman et al. (1998) reported that females with a high VO_2 max have a higher REE for their metabolic size (per kilogram of LBM) and that energy requirements are increased in endurance trained females. Lennon et al. (1984) found a significant correlation between VO_2 max and a change in REE in obese females undergoing an aerobic exercise program. However, studies which have compared the

effects of aerobic training in combination with VLCD's have conflicting results (Heymesfield et al., 1989, Phinney et al., 1982, 1992, Warwick & Garrow, 1981).

1.2.ii. Thermic Effect of Food

The thermic effect of food (TEF), also referred to as dietary-induced thermogenesis, is the energy required for the metabolism of food. TEF consists of two components; obligatory thermogenesis and facultative thermogenesis. Obligatory thermogenesis represents the energy requiring processes of digesting, absorbing and assimilating food nutrients, whereas faculatory thermogenesis represents the increase in metabolism with food ingestion related to the activation of the sympathetic nervous system (McArdle, Katch, Katch, 1996, Mole, 1990). This rapid increase in metabolic rate that occurs immediately after eating a meal may stay elevated for as long as 8 hours (Rubner, 1902). TEF accounts for approximately 7- 10 % of the total daily energy expenditure and the magnitude of variability in normal individuals depends on the quantity and type of food eaten. Pure protein meals elicit a larger thermic effect due to the greater digestive processes and the extra energy required to deaminate amino acids in the liver. This thermic effect has been reported to be as high as 25% of the total calories of the meal (McArdle, Katch, Katch, 1996). Typically, exercise performed following a mixed meal augments TEF, however, trained athletes have been reported as having a lower TEF. This lower TEF in athletes is hypothesized to represent an adaptation to conserve energy and glycogen during high intensity training (LeBlanc et al., 1984, Trembly et al., 1983).

1.2.iii. Measurement of Energy Expenditure from Physical Activity

Physical activity is defined as "any bodily movement produced by skeletal muscles that results in energy expenditure" (Caspesen et al., 1985) and it is the most variable component of total energy expenditure. Physical activity accounts for between 15 to 30 % of total energy expenditure (McArdle, Katch, Katch, 1996). Indirect calorimetry methods of determining energy expenditure include: the Douglas bag, portable respirometers, room respirometers and the ventilated hood technique. Although indirect calorimetry methods give comparable results to direct calorimetry

(Thompson 1998) they are not suitable methods to measure energy expenditure in children and adolescents. Methods of determining energy expenditure in children and adolescents have been measured by direct observation, self-report measures, heart rate and portable accelerometers (Pate, 1993). Presently, there is no gold standard for measuring physical activity in children and adolescents. The use of doubly-labelled water holds some promise, although it has not yet been validated in this population group (Pate, 1993).

1.2 iii.a Self-Report Measures of Physical Activity

Self-report measures of physical activity are the most widely used assessment technique because they are convenient, easy to administer and inexpensive (Freedson & Melanson, 1996). There are several different self-report measures of physical activity including interview-administered recalls, self-administered recalls, diaries and proxy reports. There is no standardized format for these reports as the objectives of each study varies, as well as the timeframe and amount of detailed data collection (Sallis, 1991). Although self-report measures of physical activity are easy to administer and inexpensive, they are limited by their subjective nature.

1.2.iii.b. Self-Report Activity Diaries

Annotated diaries are a direct measurement of physical activity in which records are kept on various types of physical activity performed for a specified time frame. Data collection should include a proportionate representation of weekdays and weekends as well as various seasons of the year (Montoye & Taylor, 1984). To calculate the caloric cost of an activity from activity diaries, the calories expended during rest (i.e., REE) is measured and multiplied by the MET value that corresponds with the specific activity and intensity rating. A MET is defined as a multiple of REE (McArdle, Katch, Katch, 1996), since REE is reasonably close to 1 kcal/kg body weight/min, one MET is approximately equal to 1 kcal/kg/min (Ainsworth et al. 1993). To estimate energy expenditure, body weight is multiplied by the MET value and the duration of activity. MET values for different physical activities were primarily derived from actual measurements of oxygen consumption, while others were derived from the

estimation from the energy cost of activities having similar movement patterns (Ainsworth et al., 1992).

Self-report activity diaries tend to have a high respondent burden resulting in missing or incomplete data (Montoye & Taylor, 1984) and generally provide less accurate representation of physical activity than more objective methods such as accelerometers, heart rate monitors and doubly-labelled water. Self-report methods of physical activity are especially problematic in children (Freedson, 1989) and this problem may persevere into adolescents (Pate, 1993). In addition, there are problems with misclassification of intensity levels in all ages, especially where broad intensity categories are used (Arroll & Beaglehole, 1991).

1.2.iii.c. Portable Accelerometers

Portable accelerometers have been developed as an objective measurement of physical activity, because of the limitations of self-report measures of physical activity in free-living individuals. Portable accelerometers have been reported to be reliable and objective measures of activity (Klesges et al., 1985, Maliszeewski et al., 1991, Mathews & Freedson 1995, Welk & Corbin, 1995, Epstein et al., 1996). In addition, they are of a relatively low cost (compared to doubly labeled water) and they avoid the problems of recall and subjectivity. Theoretically, when a person moves, the body is accelerated in proportion to the muscular forces responsible for the accelerations and thus to energy expenditure (Pate, 1993). The most widely used accelerometer to date has been single plane accelerometers such as the Caltrac® (Hemokinetics, Inc. Madison, WI). The Caltrac® has been validated in the laboratory setting to be a good predictor of walking energy expenditure (Maliszewski et al., 1991, Balogun et al., 1989, Montoye et al., 1983, Pambianco et al., 1990, Sallis et al., 1990). In field experiments, the Caltrac®has been observed to be useful in characterizing the activity patterns of groups of individuals (Miller et. al. 1994). Limitations of the Caltrac® include the limited ability to estimate energy expenditure associated with sedentary activities and activities which are not performed in a vertical plane (Montoye et al., 1983), difficult data retrieval, a high failure rate and a potential for tampering with the device (Williams et al., 1989).

To rectify some of the limitations of the Caltrac® accelerometer, the TriTrac-R3D® Activity Monitor (Hemokinetics, Inc. Madison, WI) has been developed. The TriTrac-R3D® is based on the same principles as the Caltrac® but can measure activity in three dimensions; horizontal, vertical, and mediolateral (Matthews & Freedson, 1995). Data is stored internally and is downloaded to a computer at the end of the measurement period. Unlike the Caltrac®, the TriTrac-R3D® has no external controls to tamper with and it can provide measurement of activity each minute for 20 days (Matthews & Freedson, 1995). Physical activity calories are estimated using a regression equation, which takes into consideration, body weight, and a summary measure of the accelerations recorded in all three vectors. This summary measure is called the vector magnitude which is calculated as the square root of the sum squared activity counts in each vector (Matthews & Freedson, 1995).

The TriTrac-R3D® and self-report measures have been found to be moderately correlated ($r = 0.46$ to 0.82) over seven days (Epstein et al., 1996, Matthews & Freedson, 1995). Welk and Corbin (1995) validated the TriTrac-R3D® using heart rate monitoring in 35, 9 to 11 year old children for three days and found a moderate correlation ($r = 0.58$). Bouten et al., (1994) found a correlation or $r = 0.95$ between a similar triaxial accelerometer and energy expenditure assessed by oxygen consumption during rest and standardized activities. Results of a recent study (Matthews & Freedson, 1995) reported the TriTrac-R3D® to significantly underestimate free-living energy expenditure compared with self-report three-day activity diaries by approximately 300 kcal/day when validated using a 7-day self-report measures. It has been suggested that the low estimation of daily energy expenditure by the TriTrac-R3D© is a result of overestimated self-reported physical activity by the subjects (Matthews & Freedson, 1995). Activity levels have been reported to be consistently greater for self-reported versus objective measures (Matthews & Freedson, 1995, Epstein et al., 1996). The TriTrac-R3D© has been found to be sensitive to relatively small changes in daily physical activity (150-200 kcal/day) (Matthews & Freedson, 1995).

2. Consequences of A Negative Energy Balance

Failure to meet energy demands in adolescents may have serious consequences on health (Thompson 1998). The health concerns associated with intense physical training at a young age has led some health professionals to suggest that training during puberty be reduced. Inadequate energy during puberty in aesthetic athletes has been found to result in nutrient deficiencies, delayed puberty, menstrual irregularities, short stature, poor bone health, and an increased incidence of injuries. Pugliese et al. (1983) studied children who restricted energy intake due to a fear of obesity and found that these children exhibited a low growth velocity, short stature and delayed puberty. Although none of these children exhibited clinical signs of AN and were not athletes, their responses to restricted energy intakes may be similar to those of aesthetic athletes.

2.1. Delayed Growth and Development

Delayed puberty (Baxter-Jones et al., 1993, Warren, 1980, Frisch et al., 1980, Malina et al., 1983, 1982, Lindholm et al., 1994) and short stature (Warren 1980, Theintz et al., 1989) are almost inevitable findings among adolescent female ballet dancers and gymnasts. Gymnasts and ballet dancers have also been reported as having a high incidence of menstrual irregularities (Lindholm et al., 1994, Warren 1980). In dancers, onset of menarche and the progression of sexual development has been correlated with a decrease in training or an injury causing forced rest for at least 2 months. Onset of menarche occurs with minimal changes in body composition and weight gain (Warren, 1980). This suggests that menarche is not body weight dependent, but more likely related to energy availability (Warren, 1980).

The normal sequence of pubertal development in ballet dancers has been reported as being reversed, with thelarche (breast development) and menarche being delayed, while pubarche (pubic hair development) is usually unaffected (Warren 1980). Normal development of pubarche and the simultaneous delay in thelarche and menarche suggest that the mechanisms responsible for triggering these events are independent. Pubarche is thought to be related to androgen secretion and higher testosterone levels, which have been reported as being elevated in female runners (Cumming et al., 1987).

There is a concern that because young aesthetic athletes have been reported to restrict energy intake, they are facing poor conditions for growth. Chronic stress and inadequate nutritional intake in combination with strenuous training can result in a negative energy balance. These factors are known to modify growth hormone, thyroid hormones and somatomedin production (Mansfield & Emans, 1993), and may therefore interfere with normal growth and development. Prolonged low levels of gonadotropins in dancers are thought to be responsible for the enhanced long bone growth, leading to eunuchoidal proportions (Warren, 1980). Warren (1980) and Dreizen et al. (1967) suggest that nutritional deprivation may be responsible for the delay in epiphysical closure and reduced skeletal growth. A marked stunting of growth in adolescent female gymnasts who trained greater than 18 hours per week before and during puberty was reported by Theintz et al. (1993) in comparison to female swimmers of similar age.

2.2. The Female Athlete Triad

The constant focus on either achieving or maintaining a prescribed body weight goal may put the young athlete at risk for developing a triad of interrelated medical disorders called the "Female Athlete Triad". The triad starts with the development of an eating disorder, which may put the athlete at risk of developing amenorrhea and subsequent osteoporosis. Premature osteoporosis is characterized by a loss of bone mass due to the hypoestrogenic effects of amenorrhea. A number of investigators have reported a significantly lower vertebral bone mineral density in young amenorrheic athletes compared with eumenorrheic control subjects (Howat et al., 1989, Drinkwater et al., 1984, Cann et al. 1984, Nelson et al. 1986, Marcus et al. 1985). The poor bone health of these athletes is most likely a combination of the effects of low calcium intakes, limited calcium absorption and menstrual irregularities. The hypoestrogenic effects of amenorrhea cause bone to become more sensitive to parathyroid hormone and as a result, a greater number of resorptive sites are established and there is a gradual loss of bone mass (Dalsky, 1990).

Aesthetic athletes may be at greater risk for low bone mineral densities as they have been reported as having inadequate energy and calcium intakes, elevated cortisol levels and menstrual irregularities (Lindholm et al., 1995, Benson et al. 1990, Bernardot

et al. 1989, Moffat 1984, Loosli 1990). Elevated cortisol may affect bone density by inhibiting bone formation, while stimulating bone resorption, and possibly lowering bone density by indirectly interfering with calcium absorption in the digestive tract and increasing urinary calcium excretion (Dueck et al., 1996a). However, despite a high prevalence of menstrual irregularities, inadequate energy and calcium intakes, gymnasts have been reported as having higher bone mineral densities than non-athlete controls and other athletes (Dyson et al., 1997, Cassell et al., 1996, Kirchner et al., 1995, Robinson et al., 1995, Nichols et al., 1994, Taaffe et al., 1995, 1997). Gymnasts expose their bones to unique mechanical forces that are considered to be high impact, which provides a high mechanical stimulus to bone mineralization (Barr & McKay, 1998). Evidence suggests that weight bearing activity during periods of growth may be maintained during adulthood as former gymnasts (Kirchner et al., 1996) and ballet dancers (Khan et al., 1997) have been reported as having higher bone mineral densities than age, height and weight matched non-athlete controls.

3. Negative Energy Balance and Menstrual Function

There is a large body of research implicating menstrual dysfunction with a negative energy balance in female athletes. The cessation of normal menstrual function is hypothesized to be an energy conserving mechanism for female athletes who are in a state of negative energy balance (Loucks et al., 1998, Brownell et al., 1987). Warren (1980) was the first to suggest that a negative energy balance as a result of intense exercise and dietary restriction resulted in an energy deficit, which leaves the body incapable of sustaining the metabolic demands of reproduction. Some suggest that it is the intense physical training which is not compensated for by increased caloric intake which causes excessive thinness, delays puberty and causes secondary amenorrhea (Warren, 1979, Frisch et al., 1980). This theory is supported by the similarities between athletic amenorrhea and anorexia nervosa and by endocrine signs of a chronic energy deficit in amenorrheic athletes (Loucks et al., 1998).

The majority of studies comparing energy intakes of eumenorrheic and amenorrheic runners report amenorrheic runners consume lower energy intakes (200 - 900 kcal lower) than their eumenorrheic counterparts (Refer to Table 2.2), with most

studies reporting similar training intensities (km of running per week) (Myerson et al., 1991, Marcus et al., 1985, Deuster et al., 1986). With the exception of Wilmore et al., (1992), amenorrheic runners reported lower caloric intakes than eumenorrheic runners. However, a significant difference between groups was only reported by Nelson et al., (1986). When caloric intake was expressed relative to body weight and composition (LBM), amenorrheic runners still consumed less than the eumenorrheic athletes. Although most studies were unable to find a significant difference between the caloric intakes of athletes and sedentary controls, it is reported that the athletes do not compensate calorically for their increased energy expenditure (Brownell et al., 1987, Wilmore et al., 1992). An intervention study by Dueck et al., (1996b) showed that hormone profiles and menstruation normalized in an amenorrheic athlete who was in a negative energy balance by reducing training and increasing daily energy intake by 300 to 400 kcal per day.

Table 2.2. Summary of Average Energy Intakes of Eumenorrheic and Amenorrheic Athletes

Study	Athletes	Energy Intakes of Eumenorrheic Athletes kcal/day (kcal/kg wt)	Energy Intakes of Amenorrheic Athletes kcal/day (kcal/kg wt)	Energy Intakes of Eumenorrheic Non-athlete Controls kcal/day (kcal/kg wt)	p Value
Cohen et al. (1985)	Ballet Dancers	1798	1384		.05
Deuster et al. (1986)	Distance runners	2489 (47.7)	2151 (42.6)		NS
Drinkwater et al. (1984)	Distance Runners	1965 (33.9)	1623 (29.8)		NS
Kaiserauer et al. (1989)	Distance Runners	2490 (45.9)	1582 (32.1)	1688 (27.4)	.05 (.001)
Marcus et al. (1985)	Distance Runners	1715 (31.9)	1272 (25.6)		NS
Myerson et al. (1991)	Distance Runners	1934 (37.8)	1730 (33.7)	1776 (29.3)	NS
Nelson et al. (1986)	Distance Runners	2250 (40.6)	1730 (30.0)		.02 (.005)
Perron & Endes (1985)	Ballet Dancers	2227	1528		.05
Wilmore et al. (1992)	Distance Runners	1690 (32.5)	1781 (34.9)	1763 (29.3)	NS

Lean athletes may be at greater risk for menstrual abnormalities because they already have low energy reserves and less tolerance for chronic negative energy balance (Alvero et al., 1998, Kurzer & Calloway, 1986). Women subjected to an energy

restriction diet (17 kcal/kg body weight) for 1 month, displayed menstrual irregularities. however, the leanest women displayed the greatest menstrual dysfunctions (Kurzer & Calloway, 1986). Researchers have studied the effects of a negative energy balance on menstrual function by mimicking the energy deficit experienced by athletes. Williams et al. (1995) examined the effect of exercise with and without energy restriction on LH secretion in eumenorrheic women over 3 consecutive menstrual cycles during the follicular phase. A significant decrease in LH pulse frequency was experienced only during periods of energy restriction and high exercise periods suggesting that LH secretion is disrupted only when subjects are in a negative energy balance as a result of an increase in training volume and energy restriction. The results of Williams et al. (1995) support the earlier research by Bullen et al. (1985), who reported a higher incidence of menstrual irregularities in women who were subjected to an intense training program plus a diet restriction component, compared to women subjected only to an intense training program. Recently, Loucks et al., (1998) reported that inadequate energy relative to energy expenditure, not the stress of exercise causes reduced LH pulsatility.

4. Negative Energy Balance and Resting Energy Expenditure

Reductions in REE may result from a negative energy balance, as it is hypothesized that REE may decrease in an attempt to conserve energy for the body's vital functions (Wade et al., 1996). Brownell et al. (1987) suggest that in response to restricted food intakes, low body weights, and a high level of energy expenditure, a phenomenon called food efficiency may increase as a protective response. Food efficiency is defined as the ratio of weight change to ingested calories (Brownell et al., 1987). It is an index of how many calories a person must eat to maintain a given weight or body composition. Brownell et al., (1987) state that "food efficiency increases when the calorie level necessary to sustain a kilogram of body weight decreases". Increased energetic efficiency is suggested to be the body's response to signals that some aspect of energy storage may be below a regulated point. Energy depletion could be prevented by increasing the efficiency of food. An increased energetic efficiency may explain stable body weight found in many endurance and aesthetic athletes despite chronic

negative energy balance (Brownell et al., 1987). Food efficiency is thought to be more pronounced in athletes who are the furthest below their ideal body weight (Brownell et al., 1987). Energy intakes of aesthetic athletes (Table 2.1) and amenorrheic runners (Myerson et al., 1991, Deuster et al., 1986a) have been reported to be either lower or similar to non-athlete controls. The endurance athletes who were amenorrheic were also reported as being weight stable despite insufficient caloric intakes needed to compensate for their high energy expenditure levels. These observations suggest that in lieu of an energy deficit, athletes may be conserving energy in other areas (Myerson et al., 1991). A lowered REE has also been associated with inadequate caloric intake as seen in chronic dieters and individuals with anorexia nervosa (Warren & Vande Wiele, 1973). Termination of ones menstrual cycle has also been suggested as an energy-conserving adaptation in response to inadequate caloric intake (Warren 1983).

Fogelholm et al. (1995) examined the hypothesis that aesthetic female athletes would have a greater energy deficit and reduction in REE compared to non-aesthetic female athletes and non-athlete controls. Gymnasts and figure skaters comprised the aesthetic athletes and soccer players represented the non-aesthetic athletes. Although differences were not significant, the aesthetic athlete group had the greatest energy expenditure, the lowest reported energy intake of the groups and the greatest energy deficit between reported energy intake and estimated energy expenditure. Nevertheless the aesthetic athletes did not show significant energy conservation by a reduction in REE when adjusted for fat free mass and fat mass. It should be noted however that this study did not state whether the phase of the menstrual cycle was controlled for during the measurement of REE. This could result in significant inaccuracies in REE as it is highest during the luteal phase and lowest during the first half of the follicular phase (Bisdee et al., 1989, Solomon et al., 1982).

5. Resting Energy Expenditure and Menstrual Function

Most of the evidence for energy conservation in the etiology of athletic amenorrhea originates from the work of Myerson et al.(1991), who investigated REE and energy balance in amenorrheic and eumenorrheic runners and eumenorrheic sedentary controls. REE was significantly lower in the amenorrheic runners in

comparison to the eumenorrheic runners and sedentary controls when adjusted for weight and LBM. A greater energy deficit was reported in the amenorrheic runners (194 kcal/day) in comparison to the eumenorrheic runners (148 kcal/day), with the controls reported as being in energy balance. Although no significant differences were found in total caloric intake between groups, there was a trend for amenorrheic runners to have lower energy intakes than eumenorrheic runners. Age, body weight, body composition, training pace, mileage, best 10 kilometer race time, years running and VO_2 max scores were similar between the two running groups and all groups were reported as being weight stable (± 2 kg) over the previous 6 months. However, the amenorrheic runners scored significantly higher on a scale of restrained eating in comparison to the eumenorrheic runners and sedentary controls. From these results, Myerson et al. (1991) conclude that the amenorrheic runners do not appear to increase their caloric intake to support their high energy expenditure levels, but instead lower their REE. The lowered REE and cessation of menstrual cycles is suggested to be an adaptive mechanism to maintain their stable weight.

It has been suggested that decreased progesterone secretion during the luteal phase may be an energy conserving process as the luteal phase has been associated with increased energy expenditure (Webb, 1986). This theory coincides with decreased ovarian function being the earliest phenomenon in the adaptation to decreased energy intake (Schweiger et al., 1987). Wilmore et al. (1992) performed a similar study and found conflicting results to those of Myerson et al. (1991). Wilmore et al. (1992) showed that energy intakes, and REE did not differ between amenorrheic and eumenorrheic runners. Several factors were suggested to contribute to the differences between these two studies. Myerson et al., (1991) reported significantly more disordered eating patterns among the amenorrheic athletes than the eumenorrheic athletes, whereas eating disorders were not screened for by Wilmore et al. (1992). Another discrepancy was that Myerson et al., (1991) reported that at least 3 of their eumenorrheic athletes may have been anovulatory, whereas no measure of ovulation was performed by Wilmore et al. (1992). Thus some of the eumenorrheic athletes in the study by Wilmore et al. (1992) may have been experiencing menstrual abnormalities despite regular menstrual bleeding. In addition, only Myerson et al. (1991) controlled

for the time during the menstrual cycle that RMR was measured in their eumenorrheic athletes. Webb (1986) suggests that the postovulatory rise in progesterone may account for a 9% increase in RMR. Thus if Wilmore et al. (1992) measured RMR during the luteal phase, this may explain higher RMR values for the eumenorrheic athletes.

Other evidence to support a reduction in RMR in amenorrheic athletes may be related to the decreased level of thyroid hormones in a state of energy restriction. Thyroid hormones have a direct effect on oxygen consumption, heat production and resting metabolic rate (Greenspan, 1997). Specifically, triiodothyronine (T_3) increases oxygen consumption and heat production in part by stimulation of Na^+-K^+ ATPase in most tissues, which contributes to an increase in REE (Goodman 1998). In general, insufficient available energy results in a reduction in thyroid hormones, as dietary intake is one of the factors controlling the deiodination process of thyroxine (T_4) to T_3. Low T_3 Syndrome, which is characterized by reduced T_3 and free-T_3 (fT_3) and elevated reverse T_3 (rT_3) during caloric restriction, has been reported in amenorrheic athletes but not eumenorrheic athletes (Loucks et al. 1992). A lower T_3 could lower REE and the caloric cost of other activities (Greenspan, 1997). Loucks and Heath (1994) reported that Low T_3 Syndrome is induced in exercising women at a threshold of energy availability. Specifically, Low T_3Syndrome is induced between 19.0 and 25.0 kcal/kg LBM/day (Loucks & Heath, 1994). Myerson et al. (1991) reported that T_3 was lower in the amenorrheic runners who also had a lower REE in comparison to the eumenorrheic runners, without a rise in rT_3 as seen in Low T_3 Syndrome. Similarly, Bosello et al. (1981) found an increase in rT_3 for a dieting group but no change for a diet plus exercise group.

VII. Summary of Literature

Several factors have been implicated in the etiology of menstrual abnormalities of female athletes. These factors include both physical and mental stress of training and competition, restrained eating, eating disorders, inadequate caloric intake, the macronutrient content of the diet and low body fat reserves. There is adequate evidence to suggest that chronic energy restriction, alone or in combination with intense training at a young age can have detrimental effects on menstrual function. Low energy

availability has been found to disrupt LH pulsatility in female athletes. Reduced LH pulsatility is a result of inhibition of GnRH at the level of the hypothalamus or higher CNS centres. Alterations in the gonadotropins may lead to inadequate follicle and corpus luteum development, compromising ovarian hormone production. Estrogen and progesterone are suppressed with chronic exercise and acute bouts of intense exercise increase the ovarian hormones, which may further inhibit LH and FSH and compromise follicle and corpus luteum development. Thus, a chronic negative energy balance plays an important role in the etiology of menstrual abnormalities, especially in individuals with already low energy reserves. This theory is supported by reports that amenorrheic athletes do not compensate for inadequate levels of energy intake relative to energy expenditure; by similarities between amenorrheic athletes and AN and by endocrine signs of chronic energy deficit in amenorrheic athletes (Loucks et al., 1998). A negative energy balance may result in nutrient deficiencies, delayed growth and development, decreased performance, menstrual irregularities, poor bone health, a lowered immune system and an increased incidence of injuries in female adolescent athletes.

The cessation of normal menstrual function is hypothesized to be an energy conserving mechanism for athletes in a state of negative energy balance. Several researchers suggest that REE and TEF may be reduced in an attempt to conserve energy for the body's vital functions at the expense of maintaining normal menstruation. This theory is supported by reports that amenorrheic athletes in a state of chronic negative energy balance have low T_3 Syndrome. More research is needed in the area of energy conservation as conflicting results exist as to whether REE is lowered. Future research should control for the timing of the menstrual cycle, and the presence of eating disorders when measuring REE. Problems in the determination of energy balance are many, as the accuracy of the measurement of energy intake and energy expenditure in free living subjects is questionable. Although adult endurance athletes have been studied extensively, little research has been conducted in adolescent aesthetic athletes. Adolescent aesthetic athletes may be more susceptible to menstrual irregularities when faced with a negative energy balance because of their extra energy requirements for growth and the added pressure for acquiring low body weight in aesthetic sports.

Intense training before the maturation of the HPO axis may also make adolescent athletes more susceptible to menstrual abnormalities. Research in this specific population is needed to determine standards and criteria for prevention and treatment of menstrual abnormalities.

CHAPTER THREE

METHODOLOGY

I. Experimental Design

This study was a descriptive cross-sectional study of three groups of young women. All groups completed the same measurements and questionnaires. Ethical approval was granted from the Faculty of Agriculture Forestry and Home Economics (Appendix I), and parental consent was required for all subjects younger than 18 years of age (Appendix II).

1. Subjects

1. Three groups of females between the ages of 15 and 18 years were recruited. Two groups of aesthetic athletes were recruited and classified according to their menstrual cycle status; oligo/amenorrheic athletes and eumenorrheic athletes. A control group of non-athlete eumenorrheic females was also recruited.
2. Eumenorrheic non-athlete controls were defined as those individuals who had menses with cycle intervals of 25 to 35 days and who participated in less than 5 hours of intense physical exercise per week and/or a relative VO_2 max less than or equal to 33 ml/kg/min (CSTF, 1986).
3. Eumenorrheic aesthetic athletes were defined as those athletes training in an aesthetic sport a minimum of 16 hours per week, who had menses with cycle intervals of 25 to 35 days (Goldfein & Monroe, 1997).
4. Oligo/amenorrheic aesthetic athletes were defined as those athletes training in an aesthetic sport a minimum of 16 hours per week, who had a cycle interval of less than 25 days or greater than 35 days (Goldfein & Monroe, 1997).
5. All participants must have reached menarche (had at least one menstrual cycle).

2. Exclusion Criteria

Individuals were excluded from the study if they were:

1. taking oral contraceptives or other medication, which would affect the menstrual cycle.
2. taking medications which would affect RMR (thyroxine, testosterone, stimulants).
3. cigarette smokers.
4. unable to provide informed consent.

3. Recruitment and Screening

Subjects were recruited through notices sent to special interest groups such as community gymnastic, figure skating and synchronized swimming clubs (Appendix III). The eumenorrheic non-athlete control group was recruited by public notification to community organizations and clubs. The subjects came to the University of Alberta, and completed the Menstrual History and Health Questionnaire and if the subjects met the inclusion criteria they were instructed to contact the principal investigator on Day 1 of their menstrual cycle. Day 1 corresponds to the first day of menses. All tests were coordinated with subjects' menstrual cycle. A random day was chosen to represent Day 1 for the athletes who were amenorrheic.

3.1. Menstrual History and Health Questionnaire (Appendix IV)

The Menstrual History and Health Questionnaire was adapted from (Harber & Sanderman, 1995) and was the primary tool used for screening. This questionnaire is a general information questionnaire related to menstrual history and overall health. The primary purpose of this questionnaire was to establish individual menstrual patterns, level of physical activity and to screen for oral contraceptive use or the use of other medications. Specific questions regarding the date of menarche, length and duration of the average menstrual cycle and general questions pertaining to physical activity, such as the number of hours spent in training per week and the number of years involved in the sport were included.

II. Methodology

1. Criteria For Establishing Menstrual Status

The menstrual status of the subjects was established by determining the length of the cycle during which the progesterone sampling took place and by self-report from the Menstrual History and Health Questionnaire. If subjects reported 10 to 12 menstrual cycles in the past 12 months, and had a cycle interval length of between 25 and 35 days for the cycle that luteal phase progesterone concentrations were determined, they were classified as eumenorrheic. Subjects who reported cycle lengths outside of these criteria were classified as oligo/amenorrheic. Subjects kept track of their menstrual cycles for the duration of the study on monthly calendars provided by the investigator. The calendars were used to help establish individual menstrual patterns.

2. Determination of Age of Menarche and Gynecological Age

Age of menarche and gynecological age were determined because they are important markers for assessing sexual maturation and normal pubertal development. Age of menarche is defined as the age at which females experience their first menstrual flow and it is the most commonly used maturity indicator (Faulkner, 1996). The recall method for assessing age of menarche involves asking females to recall to the best of their ability, the date of their first menstrual cycle either by questionnaire or interview. This method for assessing age of menarche is simple, practical and can be done at any age (Faulkner, 1996). Correlations between the actual date of menarche and the recalled age, 4 and 19 years after the event range from 0.81 to 0.78 respectively (Bergsten-Brucefors, 1976), (Damon et al., 1969). It has been reported that 63% of girls could accurately recall the date of menarche within 3 months (Bergsten-Brucefors, 1976). The subjects in this study were asked to recall their age to the nearest month that they experienced their first menstrual cycle.

Gynecological age is defined as the number of years post-menarche and is positively associated with the incidence of ovulatory menstrual cycles (Vuorento &

Huhtaniemi, 1992). Gynecological age was determined by subtracting the age at which menarche occurred from chronological age to the nearest month.

3. Pubertal Developmental Assessment

The Tanner Visual Pubertal Self-Assessment Scale (Appendix V) is a visual assessment scale used for the determination of the stages of sexual development. Sexual development was assessed because active young females sometimes have delayed sexual development and/or a reversed order of development of specific characteristics despite the presence of a menstrual cycle (Marshall & Tanner, 1969). Previous research has found that breast development and/or pubic hair growth may be delayed and the natural order of development may be reversed (Marshall and Tanner, 1969).

Tanner (1962) developed a rating system for the development of breasts and pubic hair. Each characteristic is rated on a 5-point scale. Stage 1 indicates the prepubertal stage; Stage 2 indicates the initial development of the sexual characteristic; Stage 3 and 4 indicate continued development; and Stage 5 represents the adult or mature development of the sexual characteristic. Assessing stages of sexual development in clinical studies is typically done by direct visual observation by a physician. Since there are many ethical concerns associated with this invasive method, self-assessment methods have been developed. It has been demonstrated that children and adolescents can rate their own sexual development accurately and reliably (Duke et al., 1980, Morris & Udry, 1980). Duke et al., (1980) reported kappa coefficients between physician and self-rating scores of 0.81 and 0.91 for breast and pubic hair development in females respectively.

This self-administered visual assessment scale was adapted from the Tanner physical examination method to determine stage of puberty (Marshall and Tanner, 1969). Subjects are shown pictures of each of the five stages and asked to compare and then choose which one applies to themselves in regards to breast and pubic hair development. Confidentiality of the results is emphasized, by explaining to the subjects their results are identified by number only. The self-assessment scale is then completed in a private environment. There are 5 points given for breast development and 5 points

given for pubic hair development, with each point corresponding to the stage of sexual development. Subjects are then given a puberty index score out of 10, which is the sum of breast and pubic hair stages. A score of 10 indicates the subject is in Stage 5, the last stage for both breast and pubic hair development. Breast and pubic hair development were also assessed individually to determine the developmental stage of each.

4. Anthropometrics

Body weight and height were measured twice over two months to conduct other tests which rely on these two measures such as REE, the initialization of the TriTrac-R3D® accelerometer, and the VO_2 max test. The same trained technician using standardized techniques determined anthropometric measurements. For weight and height measurements the subject was in light clothing without shoes. Body weight was measured using a beam balance scale to the nearest 0.1 kilogram. Height was measured using a set square to the nearest 0.1 centimeter with the subject standing straight with the head in the Frankurt plane, feet together, knees straight, and heels, buttocks and shoulder blades in contact with the vertical surface of the wall.

5. Sum of Skinfolds

The sum of skinfolds was used for comparison purposes among the three study groups as an indirect assessment of body fat. Skinfolds at five sites were measured using Lange calipers. The five sites included triceps, biceps, subscapular, iliac crest and the medial calf, all of which were measured on the right hand side of the body by the same trained technician using standardized techniques. Each skinfold site was measured twice, and a third time if the difference between the first two measurements was greater than 3 mm, and the mean value of the reading for each site was used. The sum of skinfolds was compared with age and gender matched norms (CSTF, 1986).

6. Aerobic Fitness Assessment

For the purpose of this study, aerobic fitness levels were determined to verify and classify the athlete groups and the non-athlete group. Fitness levels were measured by a maximal exertion VO_2 max test using a Monark cycle ergometer and a Horizon

metabolic measurement system (Sensormedics Inc., Yorba Linda, CA). This test determines maximal aerobic exercise power by measuring maximal oxygen uptake under maximal exertion conditions. The VO_2 max test is generally accepted as the best available measure of aerobic power (McArdle, Katch, Katch, 1996).

Prior to testing, subjects were required to complete a PAR-Q (CSTF, 1986) to screen for possible medical complications that would prevent the subject from completing the test (Appendix VI). The aerobic fitness testing was completed in the Exercise Physiology Lab at the Physical Education building, University of Alberta. The test involves a progressive increase in exercise intensity to the point where the subject will no longer continue to exercise. Intensity is increased by increasing the resistance on the cycle ergometer by 0.5 kiloponds every 2 minutes. Although the test typically requires the participant to exercise to exhaustion, they are ultimately in control of terminating the test. Heart rate and ergometer RPM were also monitored every minute. Both absolute aerobic power (L of O_2 /min) and relative aerobic power (mL O_2/kg/min) were recorded.

There are several objective criteria used to establish maximal effort. Maximal effort is demonstrated by a peak and levelling-off in oxygen uptake, respiratory exchange ratios in excess of 1.00, attainment of the age-predicted maximum heart rate and volitional fatigue (Freedson & Melanson, 1996). When oxygen uptake does not level-off, this may indicate that performance may be limited by muscular factors rather than cardiac capacity (McArdle, Katch, Katch, 1996). In this case, peak VO_2 is used, which reflects the highest value of oxygen uptake measured during the test.

There are several modes of exercise used for VO_2 max testing. The cycle ergometer was selected as an appropriate laboratory activity in which to compare the aerobic capacity of athletes to non-athletes, as the athlete group was not training regularly on cycle ergometers, so the task was equally familiar to all study groups. Furthermore, cycle ergometry allows for the absolute rate of work and is independent of body weight and can be tightly controlled (Freedson & Melanson, 1996). Correlation coefficients for the reliability of maximal VO_2 max tests for children range from 0.42 to 0.95 with a tendency towards 0.76 (Safrit, 1990).

7. Dietary Restraint

The Dutch Eating Behavior Questionnaire (DEBQR) was utilized to assess dietary restraint. (Appendix VII). The DEBQR consists of the last ten questions of the Eating Disorder Inventory questionnaire (a questionnaire which assesses eating disorder symptomatology). The DEBQR is in the form of a Likert scale with the following categories; never (1), seldom (2), sometimes (3), often (4), and very often (5). There is also a "not relevant" category for some of the questions. The issue of confidentiality was explained, emphasizing that only the primary investigator would have access to the information. The importance of answering the questionnaire honestly was stressed to each subject prior to the administration of the questionnaire. Subjects then completed the questionnaire in a private setting. Each question of the DEBQR was given a rating from 1 to 5 based on the response of the participant. The questionnaire was then tallied and divided by ten to give a final score. The DEBQR has a possible score ranging from 1- 5, with 1 being a very low to negligible restraint score and 5 being the highest possible restrained eating score. The DEBQR has a test-retest reliability of .92 as measured by Cronbach's α (Gorman & Allison, 1995).

8. Dietary Intake (Appendix VIII)

Dietary intakes were assessed by three-day food records on one occasion throughout the study, with the starting date ranging from Day 1 to 7 of the menstrual cycle. The food records were collected over two training days and one day of rest (measurement days did not always correspond to two weekdays and one weekend day) with the days corresponding to the same days as the energy expenditure measurements. This ensured that the total energy intake could be compared with total energy expenditure. Food records of the non-athlete controls included one weekend day in the analysis. Every precaution was taken to minimize the sources of potential error that occur with determining energy intakes. Each subject was carefully instructed how to record food intake, with particular detail in regards to serving size, amount, brand name and method of cooking. Food models, utensils, and measuring instruments were used to help explain portion sizes. It was also emphasized that subjects maintain typical

eating habits. Once completed, food records were reviewed and confirmed for accuracy with the participant.

The data collected from the three-day food records was analyzed by a computerized nutrient analysis software program, Food Processor II for Windows (Food Processor II™, Esha Research, Portland, Oregon). From the computerized analysis, energy intake in kilocalories was determined, so that a comparison could be made to the average kilocalories expended per day to establish energy balance status.

9. Total Energy Expenditure

In adults, living in Western countries, resting metabolic rate accounts for 60% to 70% of the caloric expenditure, the thermic effect of food for 5% to 10% and physical activity for 20% to 30% (McArdle, Katch, Katch, 1996). Total energy expenditure was derived from the sum of REE, the thermic effect of food (7% of REE), and activity calories. Energy expenditure due to physical activity was measured by a portable accelerometer and by self-report activity diaries when the accelerometer could not be worn. Studies that have determined total energy expenditure have either measured REE or estimated it using various prediction equations. Energy expenditure due to physical activity is usually estimated using activity diaries or heart rate monitors. Studies that have determined total energy expenditure in adolescents are minimal and the combination to determine TEE that was used in this study has not previously been used in children or adolescents (Thompson, 1998).

9.1. Resting Energy Expenditure (REE)

Resting energy expenditure was measured by indirect calorimetry using a metabolic cart (Vmax 29N, Sensormedics, Yorba Linda, CA). Measurement of REE by indirect calorimetry measures the volume of oxygen (VO_2) consumed and the volume of carbon dioxide (VCO_2) expired. Indirect calorimetry is based on the principle that adenosine triphosphate (ATP) is derived primarily from substrate oxidation, and since the oxygen stores of the body are insignificant, the volume of oxygen consumed is closely correlated with the rate of heat production and thus energy expenditure (McArde, Katch, Katch, 1996). All measurements were performed in the metabolic

testing laboratory at the University of Alberta Department of Agricultural, Food and Nutritional Sciences. All subjects followed a standardized protocol prior to testing. The test was performed between Days 1 and 7 of the menstrual cycle, corresponding to the beginning of the follicular phase, as there is an 8 to 15% increase in REE during the luteal phase due to ovulation (Webb, 1986, Bisdee & James, 1983). Subjects were required to fast and refrain from exercise for 12 hours prior to testing and to arrive at the test site by motor vehicle. The metabolic cart was calibrated against a reference mixture of oxygen (16%) and carbon dioxide (4%) gas, and body weight and height, gender and age were entered into the metabolic cart's software program. The subject was then instructed to lie on a cot in the supine position while the lights were dimmed and relaxation music was played. Subjects rested 30 minutes under these conditions, after which time the transparent hood was placed over the subject's head and the test was started.

Expired CO_2 (VCO_2) and oxygen consumption (VO_2) was determined by gas analyzers within the VMAX system. Specifically, oxygen consumption and carbon dioxide production are calculated from the differences in their concentrations in the inflowing and outflowing air and the flow rate. Subjects remained awake, but motionless, for the duration of the test. The actual test typically required a minimum of 15 minutes in order to obtain steady state measurements. Steady state occurred when the subjects attained the pre-programmed steady state conditions for minute ventilation, heart rate, VO_2, and respiratory quotient (RQ) (Sensormedics, 1995). A total test period of 20 to 40 minutes was therefore required. A RQ of 0.85 or less was considered to reflect a fasted subject.

Energy expenditure was calculated from the equation of Weir (1949) in kcal/day. To simplify this calculation, the Haldane equation is used to determine oxygen consumption (VO_2). The Haldane equation is as follows:

$$VO_2 = [(1 - FEO_2 - FECO_2) / 1 - FIO_2] * (FIO_2 - FEO_2) * VI$$

The FEO_2 value represents the fraction of oxygen in expired air whereas the $FECO_2$ value represents the fraction of carbon dioxide in expired air. VI represents the inspired volume in litres under standard conditions (37° C, ambient pressure). It is very difficult to measure the rate of nitrogen in both the inspired and expired air, so this equation

assumes nitrogen to be the same in the expired and inspired ventilation. The determination of REE typically requires the measurement of 24 hour urinary nitrogen to account for the incomplete metabolism of protein. However, the difference in REE before and after urinary nitrogen is minimal (2%) and is therefore usually excluded (SensorMedics Corporation, 1995, Cunningham 1990). For the purpose of this study, 24 hour urinary nitrogen was not measured and the REE was calculated using an adapted version of the Weir equation. The Weir equation used for the calculation of actual REE was as follows:

REE (kcal/day) = 3.9 [VO_2 (mL/min)] + 1.1 [VCO_2 (mL/min)] * 1.41

The WHO/FAO/UNO (WHO, 1985) REE prediction equation was used to compare to the measured REE. The WHO/FAO/UNO prediction equation was used as it has been found to yield average REE values that do not differ significantly from actual values measured by indirect calorimetry using the ventilated hood technique in an adolescent female Caucasian population (Wong et al. 1996, Garrel et al. 1996). The WHO/FAO/UNO REE prediction equation for females between the ages of 3 and 18 years is as follows:

REE (kcal/day) = 7.4 (Weight in kg) + 482 (Height in m) + 217

9.2. Self-Report Activity Diaries (Appendix IX)

Subjects were instructed to keep a seven-day activity diary during one menstrual cycle. Three of the seven days corresponded to the same days that energy intake was measured and the portable accelerometer was worn. The purpose of the activity diary was to establish habitual physical activity levels, and to calculate energy expenditure for the synchronized swimmers when they could not wear the portable accelerometer during their water training.

The activity diary was designed to attain information on the type of activity performed, as well as the duration and intensity of physical activity. Energy expenditure was derived from the Compendium of Physical Activities (Ainsworth et al., 1992). The Compendium of Physical Activities is a coding scheme for the classification of energy costs of human physical activities, where activities are classified by intensity and expressed as multiples of METs. The MET is based on the

principle that 5 kcal is approximately equal to 1 litre of oxygen consumed, thus one MET is equivalent to approximately 3.6 mL/kg body weight/min (McArdle, Katch, Katch, 1996). METs are also defined as multiples of REE. Since REE is approximately equal to 1 kcal/kg/min, 1 MET is approximately equal to 1 kcal/kg/min. By multiplying body weight in kilograms by the MET value and duration of the activity, energy expenditure in kilocalories can be estimated. The energy costs of the activities included in the Compendium were established from a review of published and unpublished data. The MET values for most activities were derived from actual measurements of oxygen consumption, while other activities were estimated from the energy cost of activities having similar movement patterns (Ainsworth et al., 1993). Most tables of the energy costs of physical activity are based on adult data, so substantial errors in estimating energy cost are likely if these tables are used in children. The use of METs minimizes this error (Sallis et al., 1991) because MET's take body weight into consideration.

To calculate the kilocalories expended for a given day, the number of minutes spent in each activity was converted to hours, and the time spent in a given activity level was multiplied by the appropriate MET intensity. To derive kilocalories expended during the time spent training in the water, the kcal/ kg value was summed and then multiplied by the subjects body weight in kilograms and divided by the appropriate time factor. The kilocalories obtained from this calculation were added to the kilocalories obtained from the TriTrac-R3D® and used in the calculation of the average energy expenditure per day when the TriTrac-R3D® could not be worn. The average energy expenditure per day was later used in the derivation of total energy expenditure and the energy balance equation. (See Appendix X for sample calculations).

9.3. Portable Accelerometer, TriTrac-R3D®

The TriTrac-R3D® is a three dimensional portable accelerometer that estimates energy expenditure in kilocalories due to physical activity, resting metabolism and individual patterns of physical activity (Matthews & Freedson, 1995). The TriTrac-R3D has the ability to calculate and display the calories derived from REE and

10. Determination of Energy Balance

Energy balance was determined by the net difference between energy intake and energy expenditure. In order to calculate energy balance, total energy expenditure (TEE) was first determined according to the equation below. The energy from the thermic effect of food (TEF) in the following equation was calculated as 7% of the REE.

TEE = REE (kcal/day) + TEF (kcal) + Activity Calories (kcal/day)

As previously stated, activity calories were calculated by the TriTrac-R3D® accelerometer and when the TriTrac-R3D® could not be worn by METs. A negative energy balance occurs when energy expenditure is greater than energy intake and is calculated as follows:

Negative Energy Balance = Energy Expenditure (kcal) > Energy Intake (kcal)

A positive energy balance occurs when energy intake is greater than energy expenditure and is calculated as follows:

Positive Energy Balance = Energy Intake (kcal) > Energy Expenditure (kcal)

A net difference of 500 kcal is the amount designated to represent a significant clinical difference, as this is the amount that theoretically results in a weight gain or weight loss of 0.5 kilogram per week if in a positive or negative energy balance respectively (Whitney, Hamilton, Rolfes, 1990). However for the purpose of this study, weight loss or gain was not a key outcome but rather the effects of energy balance status on luteal progesterone levels. Since the caloric deficit which results in clinical manifestations of luteal progesterone levels is unknown, no specific cut-off points were used.

11. Salivary Progesterone Measurements

Salivary progesterone measurements are a noninvasive, simple and inexpensive method of monitoring daily levels of this hormone (Ellsion, 1993). The concentration of progesterone in saliva reflects the total circulating concentrations in plasma, is independent of salivary flow rate and reflects the free fraction of the hormone (Ellsion, 1993). Morning salivary samples were obtained for measurement of progesterone from

each subject from Day 12 of the menstrual cycle until the onset of the next menses using a Salivette™ (Sarstedt, St. Laurent, PQ). Twenty-three Salivettes™ were labeled, numbered and stored in ascending order in a test tube rack for convenience and distributed to each study subject. For those subjects who were amenorrheic, salivary sampling stopped at Day 35 (maximum of 23 sampling days). Thirty-five days was the maximum cycle length for the sample collection because cycle lengths greater than 35 days are usually considered anovulatory (Highet, 1989). The Salivettes™ consisted of a test tube like container with a smaller closed test tube that encased a cotton wad. Subjects were instructed to chew on the cotton wad each morning for approximately one minute before eating or brushing teeth. The samples were then stored in a – 20° C domestic freezer until all the samples were completed. Subjects then brought the samples to the University of Alberta where salivary assays were performed by radioimmunoassay in the nutrition and metabolism laboratories.

Prior to the analysis of salivary progesterone, the sample was thawed overnight in a domestic refrigerator. The following morning the Salivettes™ were centrifuged at 600 *g* (1800 RPM) for 2 minutes to extract the saliva from the cotton wad. Progesterone concentrations were determined using GammaCoated Progesterone kit (Incstar Corportation, Stillwater, MN: catalogue # CA-1724) designed for the quantitative determination of progesterone levels in serum. This kit follows the basic principle of radioimmunoassay whereby there is a competition between a radioactive and a non-radioactive antigen for a fixed number of binding sites. The amount of ^{125}I-labelled progesterone bound to the antibody on the rabbit anti-progesterone coated tube is inversely proportional to the concentration of progesterone present in the sample being analyzed. The separation of free and bound antigen is achieved by aspirating or decanting the antibody coated tubes.

The following modifications were added to the protocol originally supplied by the manufacturer to account for the lower level of progesterone present in saliva compared to serum. Two reference controls of 0.5 ng/ml and 5.0 ng/ml of progesterone were provided and used as quality control. To increase the sensitivity of the standard curve, the provided standards were diluted with the progesterone serum blank to give concentrations of 0.075, 0.15, 0.3, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0 ng/ml. Secondly, the

volume of saliva taken to the assay was increased to 200μL and all other tube volumes were brought to 200 μL by adding 100 μL of water. Start-up testing showed no difference in percent specific bound between water and serum blank. Two non-specific bound (NSB) tubes were used to test the ability of the antibody to bind to the tracer.

For each subject's day of saliva collection, 200μL aliquots were added in duplicate, after which the progesterone tracer was added. Each test tube rack was gently shaken manually for 30 seconds and incubated in a water-bath at 37^0 C for 100 minutes, after which all tubes (except the tubes labelled as Total Counts) were aspirated. Tubes were counted by a gamma counter for 2 minutes. A new standard curve was prepared for every 200 samples. Intra- and inter-assay coefficient of variation's were 8.5 and 5.3 % respectively and average sensitivity estimated as 85 % of total binding was 0.015 ng/tube (0.075 ng/ml).

The mean progesterone concentration was determined for each subject and the results were analyzed as group means. As well, each subjects' progesterone profile is displayed in Appendix XIV. If a subject missed more than four consecutive days of sampling, the data was excluded from the group analysis.

12. Statistical Analysis

All statistical analysis were performed by the software program, Statistical Package for the Social Sciences (SPSS, Version 7.5). The three study groups were compared for differences in luteal salivary progesterone levels, energy intake, activity kilocalories, total energy expenditure, energy balance, REE, dietary restraint, pubertal development, and anthropometrics. Differences in the preceding variables were determined using one-way ANOVA and post hoc analysis by Tukey. The Levene test was used to test the homogeneity of variances among the three groups. Variables with unequal variances were compared by ANOVA and post hoc analysis by Dunnett T3. Student t-tests were used to compare the number of years that the two athlete groups were training and the age at which they started competing. Differences were considered statistically significant when p values were ≤ 0.05. Thirteen subjects per group are theoretically required to detect a statistically significant difference between salivary progesterone

concentrations (See Appendix XII for sample size calculations). All results were presented as mean ± standard deviation.

Pearson r correlation coefficients were used to determine association between the variables discussed in the hypotheses (Chapter 1), and are as follows: Mean luteal phase salivary progesterone was correlated with anthropometrics, body composition, gynecological age, age of menarche, puberty index, restrained eating, energy intake, activity calories, REE, total energy expenditure and energy balance. REE was correlated with energy balance, restrained eating (Chapter 1 hypotheses) as well as with the sum of skinfolds, number of hours training per week and VO_2 max score (refer to Chapter 2, section 1.2.i.a Factors affecting REE). Indices of pubertal development were correlated with variables hypothesized to influence pubertal development (Refer to Chapter 2). Indices of pubertal development were correlated with body weight, sum of skinfolds, number of hours training per week, VO_2 max score, dietary restraint score and energy intake. Only those correlations found to be significantly correlated at the 0.05 or 0.01 level of significance are reported.

CHAPTER FOUR

RESULTS

I. Subject Characteristics

Twenty-nine participants volunteered to be involved in the study. Ten gymnasts, eight figure skaters and three synchronized swimmers were recruited and classified by menstrual status according to the previously described criteria. The final groups were comprised of ten oligo/amenorrheic aesthetic athletes, eleven eumenorrheic aesthetic athletes and eight eumenorrheic non-athlete controls. There are incomplete data for one non-athlete control. The representation of athletes in each menstrual cycle category is depicted in Table 4.1.

Table 4.1. Type of Athlete in Menstrual Status Groups

Athlete	Oligo/amenorrheic Athletes n = 10	Eumenorrheic Athletes n = 11
Gymnasts	5	5
Figure Skaters	3	5
Synchronized Swimmers	2	1

Results are reported as number of cases per group.

The characteristics of the three groups of subjects are depicted in Table 4.2. Chronological age and dietary restraint scores were similar for all three groups. The remaining physical characteristics of the non-athlete controls were significantly different from the two athlete groups. The physical characteristics of the two athlete groups were similar. The two athlete groups had significantly lower body weights, lower skinfold measurements, and were smaller in stature than the non-athlete controls. Dietary restraint scores were higher in the two athlete groups, but were not significantly different than the scores of the non-athlete controls. The two athlete groups were more

physically fit than the non-athlete controls, and were classified as having excellent aerobic fitness in comparison to the non-athlete controls, which were classified as having poor aerobic fitness. The number of years spent training and the age at which the athletes started competing were similar between the two athlete groups.

Table 4.2. Subject Characteristics

Variable	Oligo/amenorrheic Athletes n = 10	Eumenorrheic Athletes n = 11	Eumenorrheic Non-Athlete Controls n = 8	P value
Age (year)	15.7 ± 0.67	16.4 ± 1.04	16.4 ± 1.06	NS
Height (cm)	159.2 ± 9.13 [a]	160.4 ± 4.13 [a]	171.6 ± 5.92 [b]	.001
Weight (kg)	52.8 ± 11.7 [a]	53.1 ± 4.9 [a]	65.8 ± 6.4 [b]	.004
Sum of 5 Skinfolds (mm)	53.3 ± 14.8 [a]	60.1 ± 9.4 [a]	101.7 ± 31.7 [b]	.000
VO_2 Max (ml/kg/min)	44.0 ± 5.5 [a]	44.1 ± 6.0 [a#]	31.7 ± 3.6 [b#]	.000
Years Training in Sport*	7.1 ± 2.2	7.9 ± 2.2		NS
Age started competing*	9.2 ± 1.8	10.0 ± 3.1		NS
Hours Training/week	18.9 ± 2.9 [a]	18.7 ± 2.7 [a]	1.0 ± 1.5 [b]	.000
Dietary Restraint Score	2.8 ± 1.1	2.7 ± 1.0	2.2 ± 0.5	NS

Results are presented as mean ± SD.
Variables with the same letter are not significantly different (ANOVA/Tukey)
*Variables analyzed by Student t tests.
Sum of 5 Skinfolds = biceps + triceps + subscapular + iliac crest + medial calf
Dietary Restraint score is out of a possible score of 5
[#] Incomplete data for VO_2 max score for eumenorrheic athletes, n = 9 and for non-athlete controls, n = 7

II. Pubertal Development Characteristics

The mean age of menarche of the oligo/amenorrheic athletes was significantly greater than the mean age of menarche of the non-athlete controls (Refer to Table 4.3). The oligo/amenorrheic athletes had a significantly greater gynecological age than the non-athlete controls, although not significantly greater than the eumenorrheic athletes. The puberty index score (combination of both breast and pubic hair development) of the oligo/amenorrheic athletes and the eumenorrheic athletes was significantly less than the puberty index score of the non-athlete controls. Self-report breast development was significantly less among the oligo/amenorrheic athletes than the non-athlete controls but not significantly different than the breast development of the eumenorrheic athletes. Self-reported pubic hair development of the oligo/amenorrheic athletes was significantly lower than the reported pubic hair development of the non-athlete controls. but not different from the eumenorrheic athletes.

Table 4.3. Pubertal Development Characteristics

Variable	Oligo/amenorrheic Athletes	Eumenorrheic Athletes	Non-Athlete Controls	p-value
Age of Menarche (year)	13.8 ± 1.1^{a}	13.7 ± 1.1^{a}	12.1 ± 0.8^{b}	.004
Gynecological age (year)	2.0 ± 1.6^{a}	3.0 ± 1.4^{ab}	4.3 ± 1.3^{b}	.008
Puberty Index	8.3 ± 1.3^{a}	9.1 ± 0.8^{a}	10.0 ± 0^{b}	.003
Breast Development	4.0 ± 0.7^{a}	4.4 ± 0.7^{ab}	5.0 ± 0^{b}	.004
Pubic hair Development	4.3 ± 0.8^{a}	4.7 ± 0.5^{ab}	5.0 ± 0^{b}	.043

Results are presented as mean ± SD. Variables with the same letter are not significantly different (ANOVA/Tukey). Puberty Index is out of a possible score of 10 for both breast and pubic hair development. Breast and Pubic hair Development (from Puberty Index) are each out of a possible score of 5, with 5 being fully developed.

II. Components of Energy Balance

1. Energy Intake

Energy intake was not significantly different among the three groups (Table 4.4). Energy intake per kilogram body weight was also not significantly different among the three groups. The macronutrient composition of the diet of the two athlete groups was significantly different compared to the non-athlete controls, with the exception of protein (Table 4.5). The protein content of the all three groups represented between 12 and 14% of total energy intake. The two athlete groups consumed a significantly greater percentage of energy from carbohydrates (approximately 63%) and significantly less energy from fat (approximately 25%) compared to the non-athlete controls (approximately 51% and 35% respectively). For individual results on energy balance data refer to Appendix XIV. The day-to-day energy variability within subjects (intra-assay coefficient of variation) for the three days of energy intake was 16% .

2. Energy Expenditure

With the exception of activity calories derived from the TriTrac-R3D®, there were no significant differences between the groups on any of the variables specifically used to calculate energy balance. Both athlete groups expended significantly more calories from physical activity than the non-athlete controls. There were no significant differences among the three groups for resting energy expenditure, thermic effect of food or total energy expenditure. Relative resting energy expenditure (REE/kg body weight) was significantly lower among the non-athlete controls compared to the oligo/amenorrheic athletes with no differences between the eumenorrheic athletes and the non-athlete controls. Total energy expenditure was also significantly greater among the oligo/amenorrheic athletes in comparison to the non-athlete controls.

Although not significantly different, oligo/amenorrheic athletes were in negative energy balance (-290 ± 677 kcal/day), eumenorrheic athletes were approximately in energy balance (- 5 ± 460 kcal/day) and the non-athlete controls were in positive energy balance (179 ± 592 kcal/day). There was no evidence of energy conservation in the athlete groups, in that resting energy expenditure, thermic effect of food, total energy

expenditure and the energy balance values were not significantly different among the three groups. Table 4.4, 4.5 and Figures 4.1 and 4.2 summarize the results for the components of energy balance for the three groups.

Table 4.4. Components of Energy Balance

Variable	Oligo/amenorrheic Athletes n = 10	Eumenorrheic Athletes n = 11	Non-Athlete Controls N = 8	p value
Energy Intake (kcal/d)	1911 ± 655	2076 ± 401	2105 ± 618	NS
Energy Intake (kcal/kg)	37.8 ± 14.2	38.6 ± 8.0	31.7 ± 9.8	NS
REE (kcal/d)	1465 ± 271	1428 ± 159	1564 ± 79	NS
REE % Predicted	103 ± 11	101 ± 10	101 ± 5	NS
REE (kcal/kg)	28.2 ± 4.1[a]	27.1 ± 3.7[ab]	23.9 ± 4.1[b]	.042
TEF (kcal/d)	103 ± 19	100 ± 11	109 ± 6	NS
Activity (kcal/d)	633 ± 302[a]	543 ± 169[a]	252 ± 70[b]	.002
TEE (kcal/d)	2201 ± 563[a]	2071 ±187[ab]	1926 ± 105[b]	NS
Energy Balance, (kcal/d)	- 290 ± 677	- 5 ± 461	179 ± 592	NS

Results are presented as mean ± SD. Variables with the same letter are not significantly different (ANOVA/Tukey). % Predicted = measured REE/predicted REE. Predicted REE using WHO/FAO/UNO equation, (WHO, 1985). REE (kcal/kg) = Measured Resting Energy Expenditure per kg body weight
TEF (kcal/d) = Thermic Effect of Food (7% of measured REE).
Activity (kcal/d) = Activity Calories from TriTrac-R3D©.
TEE (kcal/d) = Total Energy Expenditure = REE + TEF + Activity
Energy Balance (kcal/d) = Energy Intake – Total Energy Expenditure

Table 4.5. Macronutrient Breakdown

Macronutrient	Oligo/amenorrheic Athlete n = 10	Eumenorrheic Athlete n = 11	Non-Athlete Controls n = 8	p Value
% Carbohydrate	63 ± 7^{a}	$63 \pm 9_{a}$	51 ± 6^{b}	.001
% Protein	12 ± 2	14 ± 2	14 ± 1	NS
% Fat	25 ± 6^{a}	25 ± 6^{a}	35 ± 5^{b}	.001

Variables are presented as mean ± SD. Variables with the same letter are not significantly different (ANOVA/Tukey).

Figure 4.1. Components of Energy Balance

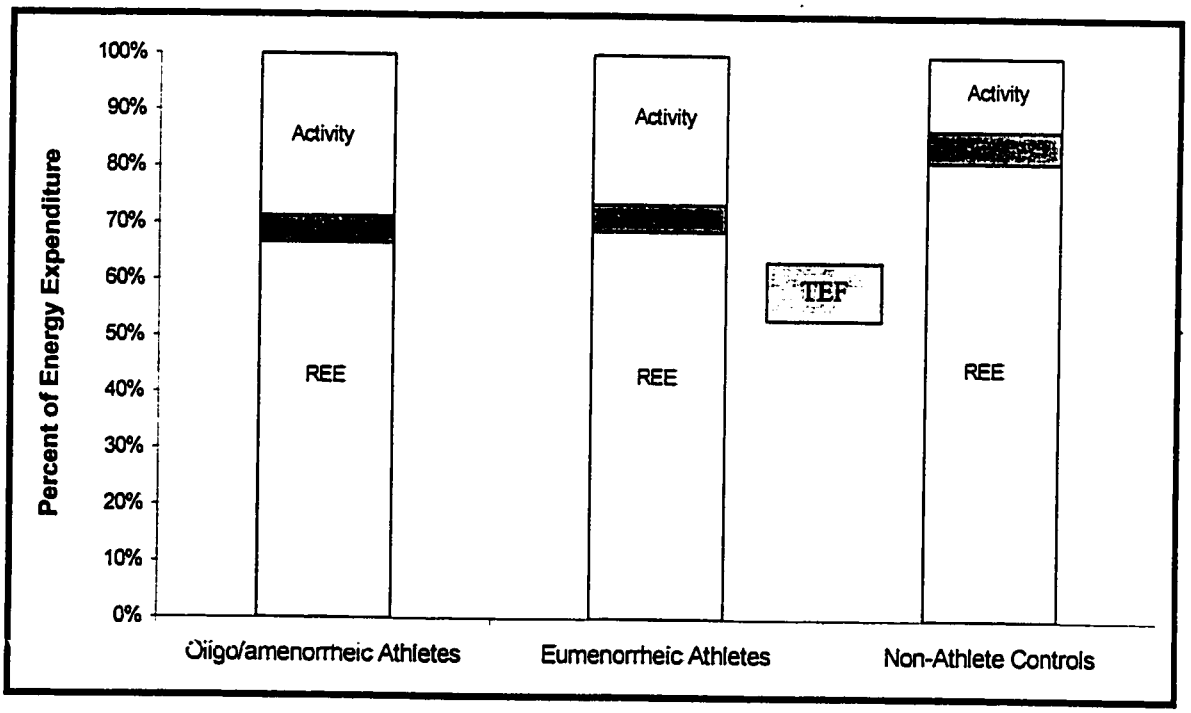
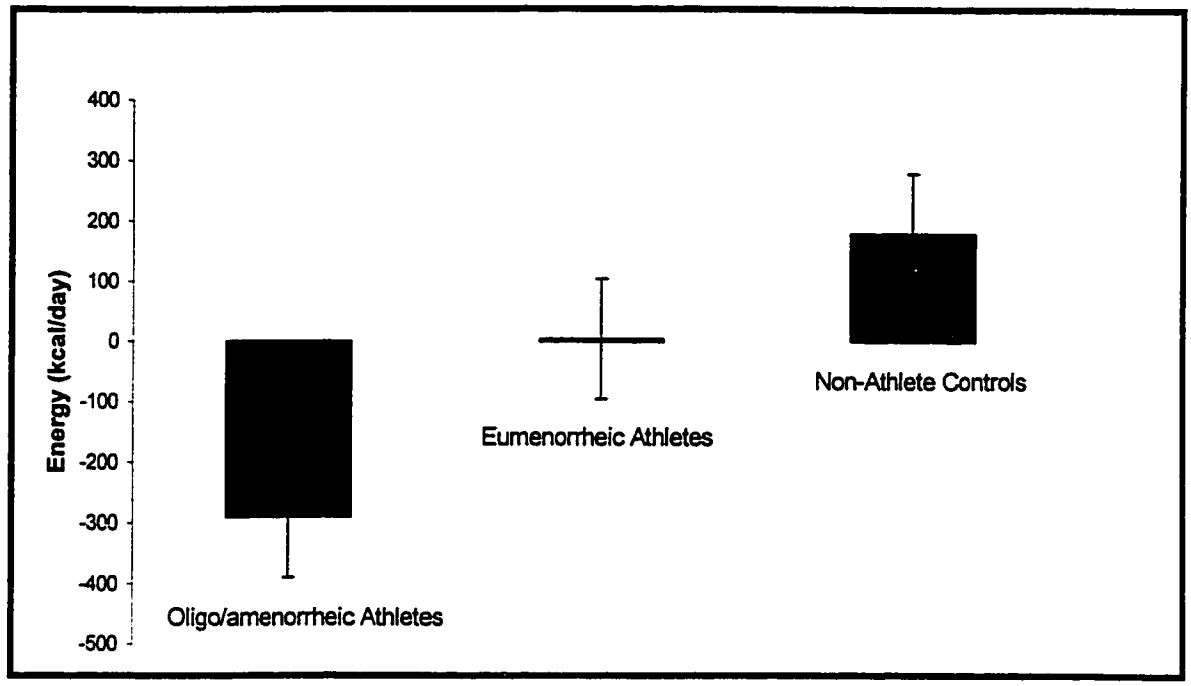


Figure 4.2. Energy Balance (Mean ± SE)



IV. Menstrual Cycle Characteristics

1. Menstrual Cycle Length

Of the oligo/amenorrheic athletes who were cyclic, menstrual cycle length ranged from 19 to 100 days. Of the ten oligo/amenorrheic athletes, four were amenorrheic. Although all of the oligo/amenorrheic athletes had reached menarche, three had only ever had one menstrual period (their menarcheal menstrual cycle). One of the athletes who was previously cyclic had secondary amenorrhea. The representation of athletes classified as amenorrheic and oligomenorrheic is depicted in Table 4.6. Menstrual cycle length ranged from 25 to 35 days among the eumenorrheic athletes and from 28 to 35 days among the non-athlete controls. Refer to Appendix XIV for individual data on menstrual cycle length.

Table 4.6. Classification of Oligo/amenorrheic Athletes

Athlete	Amenorrheic n = 4	Oligomenorrheic n = 6
Gymnasts	3	2
Figure Skaters	0	3
Synchronized Swimmers	1	1

Results are presented as number of cases in each category.

2. Salivary Progesterone Analysis

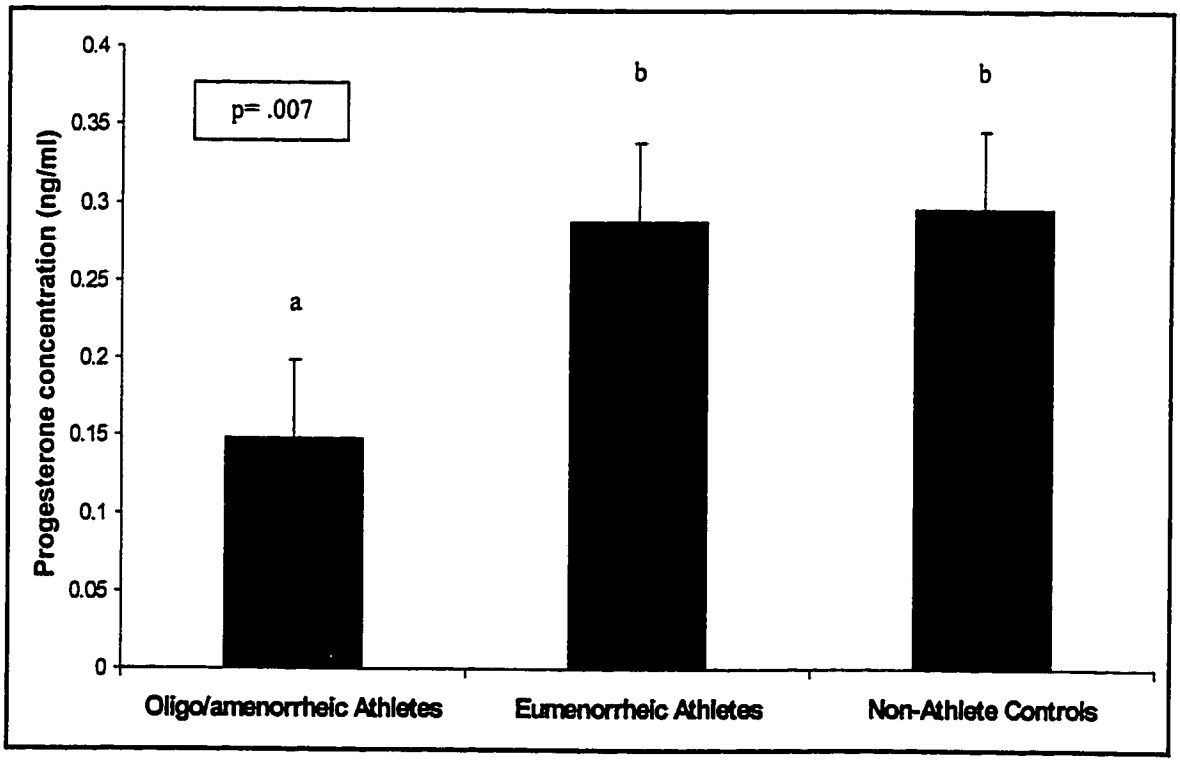
The study's protocol necessitated that if a subject missed more than four consecutive days of sampling, the progesterone data would be excluded from the group analysis. The compliance of the saliva collection by the study subjects was excellent. With the exception of one non-athlete control, no other subject missed more than for consecutive days and the missed days of all but the one non-athlete control were included as part of the mean progesterone analysis. (Refer to Appendix XIV for individual results). Mean luteal phase salivary progesterone concentrations were significantly lower in the oligo/amenorrheic athletes compared to the eumenorrheic athletes and non-athlete controls, with no differences among the eumenorrheic athletes and the non-athlete controls. Peak progesterone concentration was not significantly different among the three groups. Refer to Table 4.7. and Figure 4.3 for the results of the progesterone analysis. For individual progesterone profiles and the number of samples collected for each subject refer to Appendix XIV.

Table 4.7. Salivary Progesterone Analysis

Variable	Oligo/amenorrheic Athletes n = 10	Eumenorrheic Athletes n = 11	Non-Athlete Controls n = 7	p value
Mean luteal phase salivary progesterone concentration (ng/ml)	$0.148 \pm .007^{a}$	$0.288 \pm .103^{b}$	$0.296 \pm .134^{b\#}$	.007
Peak progesterone concentration (ng/ml)	$.304 \pm .103^{a}$	$.532 \pm .300^{b}$	$.500 \pm .216^{ab\#}$	NS

Results are presented as mean ± SD. Variables with the same letter are not significantly different (ANOVA/Tukey).
Incomplete data for non-athlete control, n = 7.
Peak progesterone is the highest progesterone concentration reached over the luteal phase.

Figure 4.3. Mean Salivary Progesterone Concentration (Mean ± SE)



IV. Pearson Correlations of Selected Variables

Variables that were analyzed by Pearson correlations are discussed in Chapter 3 (Section 12. Statistical Analysis). Only those correlations found to be significantly correlated either at the 0.05 or 0.01 level of significance are reported below.

Table 4.8. Pearson Correlations

	r	p
REE/kg (kcal/kg/day)		
Hours training/ week	.450	0.05
Sum of skinfolds	-.555	0.01
VO_2 max score	.409	0.05
Age of Menarche		
Body weight	-.613	0.01
Hours training per week	.525	0.01
Sum of skinfolds	-.559	0.01
Puberty Index		
Age of menarche	-.531	0.01
Gynecological age	.575	0.01
Body weight	.655	0.01
Sum of skinfolds	.506	0.01
Hours training/ week	-.487	0.01
VO_2 max score	-.420	0.05
Breast development		
Gynecological years	.381	0.05
Body weight	.502	0.01
Sum of skinfolds	.443	0.01
Hours training/week	-.561	0.01
VO_2 max score	-.509	0.01
Activity calories	-.370	0.05
Pubic hair development		
Gynecological age	.636	0.01
Body weight	.650	0.01
Sum of skinfolds	.439	0.05

CHAPTER FIVE

DISCUSSION

I. Major Findings

Findings that were in accordance with the study's hypotheses include the significantly lower luteal phase progesterone levels of the oligo/amenorrheic athletes compared to the eumenorrheic athletes and the non-athlete controls. Although not significant, the oligo/amenorrheic athletes had smaller sum of skinfolds, a greater dietary restraint score and negative energy balance than the eumenorrheic athletes and the non-athlete controls. The results of this study suggests that the low luteal phase progesterone concentrations of the oligo/amenorrheic athletes were not related to training since both athlete groups had been training the same amount of years, the same number of hours per week and had started competing at the same age. It was hypothesized that the oligo/amenorrheic athletes would show evidence of energy conservation by a reduced REE, as a result of a negative energy balance, restrained eating and reduced luteal phase progesterone levels. However, the results of this study do not support this hypothesis because REE was similar for all three groups and was not associated with energy balance, dietary restraint scores and luteal phase progesterone levels.

The two athlete groups were significantly lighter, leaner, more fit and reached menarche significantly later than the non-athletes. The oligo/amenorrheic athletes were found to have a significantly younger gynecological age than the non-athlete controls and although not significant a younger gynecological age than the eumenorrheic athletes. The oligo/amenorrheic athletes had a significantly slower pubertal maturation of the secondary sex characteristics compared to the non-athlete controls.

II. Subject Characteristics

Physical characteristics of the two athlete groups were similar. As expected by the design of the study, the two groups of athletes were considerably more fit compared to the non-athlete controls. A VO_2 max score greater than 43 ml/min/kg is classified as

excellent aerobic fitness for 15 to 19 year old females (CSTF, 1986). The athlete groups were classified as having excellent aerobic fitness and the non-athlete controls were classified as having poor aerobic fitness (CSTF, 1986). Athletes were significantly shorter, lighter and leaner than the non-athlete controls, which is expected considering the athletes were involved in aesthetic sports, which emphasizes a low body weight and leanness. Nutrition Canada (1980) classified all three groups as being in the 50th percentile for weight for height suggesting that all three groups of subjects were at a good weight for their height. In contrast, the sum of skinfolds classified the non-athlete controls as being at a health risk for the diseases associated with obesity (CSTF, 1986). Refer to Table 5.1 for age and gender matched norms for sum of skinfolds and VO_2 max scores.

Table 5.1. Age and gender matched norms for sum of skinfolds and VO_2 max scores (CSTF, 1986)

Percentiles for females 15 to 19 years of age	Sum of 5 Skinfolds (mm)	VO_2 max Scores (ml/min/kg)
95th percentile	36	45
75th percentile	49	41
55th percentile	58	38
35th percentile	69	36
15th percentile	89	34
5th percentile	116	32
Oligo/amenorrheic athletes	53.3	44.0
Eumenorrheic athletes	60.1	44.1
Non-athlete controls	101.7	31.7

Sum of 5 skinfolds includes triceps, biceps, subscapular, suprailiac, and medial calf.

There were no differences between the two athlete groups in terms of training. Both groups of athletes started competing at the same age and had been training the same duration. The number of hours trained per week was not different for the athlete groups, with both groups training slightly more than 18 hours per week. It is therefore not surprising that the athletes were significantly lighter, leaner and had a greater aerobic fitness than the non-athlete controls. Many investigators have suggested that delayed puberty and a reduction in growth potential may occur with restricted energy

intakes and/or intense training (Pugliese et al., 1983, Warren, 1980). Training 18 hours per week has been found to be the threshold level at which pubertal development and a reduction in growth potential has been found in adolescent gymnasts who train before and during puberty (Tanner, 1986, Theintz et al., 1993). The number of hours trained per week may have had an impact on the later maturation of the athletes as the number of hours trained per week was negatively correlated with gynecological age, puberty index and breast development and positively correlated with the age of menarche.

The finding of significantly shorter stature among the athlete group is not surprising since gymnasts comprised half the athlete group; gymnasts are typically shorter than the average female population (Benardot & Czerwinski, 1991, Claessens et al., 1992, Theintz et al., 1992). In a study of elite dancers, Brooks-Gunn and Warren (1988) found that menarche in dancers was later than their mothers and sisters, suggesting that age of menarche is not only influenced by genetics but also by intensive training. The results of this study suggest that the volume of training may negatively influence growth and pubertal maturation. However, it is premature to assume the athletes in this study were less developed and shorter compared to the non-athletes, as a result of their intensive training regimes. A more plausible explanation is that the athletes represented a select group who self-selected their sport because they already had the characteristic body type of gymnasts and figure skaters. Peltenburg et al., (1984) has shown that 8 to 14 year old gymnasts were shorter and had been shorter than the non-athletic population even prior to their participation in gymnastics. Thus, parental characteristics such as parental height and maternal age of menarche may have also influenced the later pubertal development and shorter stature in this athlete population.

III. Pubertal Development

Demographic data suggests that the age of menarche has decreased in the Western Hemisphere as lifestyles and nutrition have improved (Warren, 1983). The mean age at menarche in the United States has plateaued at 12.9 years (Roy, 1991). Athletes such as ballet dancers, gymnasts, figure skaters and volleyball players have been reported as having the latest mean ages at menarche with a greater percentage of

late maturing athletes at the more advanced levels of competition (Malina, 1982, 1983, Classens et al., 1991, Brooks-Gunn & Warren, 1988). The two athlete groups reached menarche (13.8 ± 1.1 years for the oligo/amenorrheic athletes, 13.7 ± 1.1 years for the eumenorrheic athletes) significantly later than the non-athlete controls (12.1 ± 0.8 years), which is a consistent finding among athletes in comparison to non-athletes (Warren 1980). The age at menarche for the non-athlete controls in this study are in close agreement with that reported of others. Vuorento & Huhtaneimi (1992) reported a mean age at menarche for non-athletes to be 12.6 ± 1.0. The oligo/amenorrheic athletes in this study had a significantly younger mean gynecological age (2.0 ± 1.6 years) than the non-athlete controls (4.3 ± 1.3 years). Although the eumenorrheic athletes had a younger gynecological age (3.0 ± 1.4 years) than the non-athlete controls, it was not significantly different. Results from this study and others suggest that aesthetic athletes training intensely at a young age are older at menarche compared to non-athlete controls.

The later age of menarche which is characteristic of athletes participating in aesthetic sports has been attributed to the effects of intense training before the onset of puberty. It is unknown whether it is the effects of the physical and mental stress of intense training or whether it is the energy draining effect of intense exercise or a combination of the two. It has also been suggested that menarche in these athletes may not actually be delayed from the effects of early childhood intensive training, but that it is just genetically late. Baxter-Jones et al. (1993) investigated the relationships between duration of training and menarche while also taking into consideration family and social characteristics such as maternal menarcheal age and socioeconomic status in gymnasts, swimmers and tennis players. When maternal menarcheal age, the duration of training, type of sport and socioeconomic status were assessed using analysis of covariance, maternal menarcheal age, and type of training were found to be the best predictors of the subject's age of menarche. Gymnasts were found to be the only athlete group with delayed menarche, and the amount of training did not have any effect on menarcheal age in any of the athlete groups. Significant differences between the mothers' and daughters' age of menarche were reported for the gymnasts and tennis players only. The authors suggested that genetic predisposition and intense physical training are not

the only factors affecting menarche. Gymnasts may self select the sport and the late maturation of gymnasts may contribute to their decision to continue participation in the sport as well as success in the sport.

The appearance of the secondary sex characteristics, skeletal growth, and menarche are events associated with puberty. Several studies have reported biological maturation to be slower than normal in female gymnasts (Claessens et al., 1991, Lindholm et al., 1994, Theintz et al., 1993, Malina, 1983) and ballet dancers (Warren, 1980, Frisch et al., 1980, Brooks-Gunn et al., 1987, Malina, 1983). Slower maturation in our aesthetic athletes was in accordance with these studies. Pubertal development assessed by the Tanner Visual Pubertal Self-Assessment Scale was self-reported to be significantly lower among the two athlete groups compared to the non-athlete controls suggesting that athletes have delayed or later sexual maturation of the secondary sexual characteristics. Although not significant, the stage of pubertal development was self-reported as lower in the oligo/amenorrheic compared to the eumenorrheic athletes.

Reversal of the natural order of sexual development despite the presence of a normal menstrual cycle has been reported among adolescent aesthetic athletes (Marshall & Tanner, 1969, Warren, 1980). Breast budding is usually the first sign of puberty, which occurs between the ages of 8 and 13 years for most females. Peak height velocity is reached soon after breast budding occurs and menarche usually occurs approximately 2.5 years after the initial breast budding (Roy, 1991). Pubic hair growth usually occurs after the initial breast budding but the last stage of pubic hair growth is completed before the last stage of breast development. The appearance of pubic hair may occur prior to breast budding in about one-third of all females (Roy, 1991).

Breast development (thelarche) was reported to be stage 4.0 ± 0.7 for the oligo/amenorrheic athletes, 4.4 ± 0.7, for the eumenorrheic athletes and 5.0 ± 0 for the non-athlete controls. Breast development of the oligo/amenorrheic athletes was significantly lower than the breast development of the non-athlete controls, and was given a lower Tanner stage by the two athlete groups than pubic hair development (pubarche) Tanner stage. The Tanner stage of pubic hair development of the oligo/amenorrheic athletes' (4.3 ± 0.8) was significantly lower than the Tanner stage of pubic hair development of the non-athlete controls (5.0 ± 0). The pubic hair

developmental stage of the eumenorrheic athletes (4.7 ± 0.5) was similar to the non-athlete controls. This data suggests that the oligo/amenorrheic athletes may have delayed development of the secondary sexual characteristics, particularly breast development, compared to age matched non-athlete females. Warren (1980) found similar results in a group of elite adolescent ballet dancers, who had reasonably normal pubarche and a significant delay in thelarche. Since pubarche seems to be unaffected, it is suggested that the mechanisms affecting pubarche and thelarche are unrelated. Warren, (1980) suggest that pubarche may be related to androgen secretion as greater testosterone levels have been reported in female runners (Dale et al., 1979). The mechanism for elevated testosterone levels is unclear, although it is speculated that it is related to decreased levels of body fat, where the conversion of testosterone to estrone takes place. Although pubic hair development was more developed than breast development among the two athlete groups, it is premature to conclude that the natural order of development was reversed. The initial stage of breast development usually occurs before the initiation of pubic hair development, however, the last stage of pubic hair growth is completed more rapidly than breast development (Roy, 1991). Thelarche takes considerably more time than pubarche; the mean interval of time to proceed from stage 2 breast development to stage 5 is 4.5 years (Roy, 1991).

IV. Energy Balance Components and Evidence of Energy Conservation

1. Energy Intake

Energy requirements of children and adolescents are estimated based on the observed intakes of large samples of healthy adolescents growing normally and they are average energy requirements (Health and welfare Canada, 1983). All groups' estimated energy intake fell slightly below the average energy requirements based on Canada Health and Welfare (1983) of 2200 kcal/day for females 13 to 15 years and 2100 kcal/day for females 16 to 18 years. The average energy requirements of Canadian females 13 to 15 years of age is 46 kcal per kilogram body weight and 40 kcal per kilogram body weight for 16 to 18 year old females. Thus the energy intake per kilogram body weight also fell slightly below the average recommendations of Health

and Welfare Canada (1983). The two athlete groups were within the recommendations of consuming less than 30% of total energy from fat, whereas the non-athletes did not meet this recommendation (Health and Welfare Canada, 1983). The two athlete groups derived significantly more energy from carbohydrates than the non-athlete controls. There were no differences in terms of the macronutrient contribution between the two athlete groups and the contribution of energy from protein was similar for all three groups. Thus it does not appear that the different progesterone concentrations among the two athlete groups was attributed to differences in macronutrient consumption.

Although there was a tendency for the oligo/amenorrheic athletes to consume less calories, there were no significant differences among the three groups. It is speculated that the two athlete groups would be even further below the average recommendations for energy intake because of the significantly greater amount of calories expended during physical activity. Although the nutritional status was not assessed, the athletes could have a compromised nutritional status as a result of low energy intakes. The oligo/amenorrheic athletes had a significantly lower puberty index indicative of a body still in the process of maturation and extra energy requirements would be required for the rapid growth and development that occurs during puberty for this group. This could possibly put them even further below estimated energy requirements. Strenuous training and low energy intakes during puberty are implicated in slowing or delaying the process of maturation (Roy, 1991, Warren, 1983).

Energy intake relative to body weight (kcal/kg) was not significantly different among the groups. Thus, it does not appear that energy efficiency was enhanced among the oligo/amenorrheic athletes, as they did not require fewer calories to maintain each kilogram of body weight. This finding is surprising and does not show evidence of energy conservation. It can be assumed that the athletes may be consuming adequate calories for their smaller body size and by maintaining lower than normal body weights, their energy requirements are reduced. Since energy utilization may be influenced by hereditary (Health and Welfare Canada, 1983), the athletes may have self selected their sport because they may utilize energy more efficiently than the non-athletes.

Restrictive dieting and low energy intakes are common among aesthetic athletes (Sundgot- Borgen, 1993) and have been implicated in the etiology of menstrual

dysfunction (Barr et al., 1994, Schweiger et al., 1992). Although dietary restraint scores were higher among the two athlete groups compared to the non-athletes, no significant differences existed among the groups. It is important to consider extreme cases of individuals with high dietary restraint scores when analyzing data. Refer to Appendix for XIV for individual data. Athletes participating in aesthetic sports and patients with AN have been found to have similar attitudes and behaviours (i.e. food restriction) towards maintaining a low body weight (Bale et al., 1996). However, compared to the norm for dietary restraint scores of the DEBQR for American non-obese female college students: 2.9 ± 1.0 (Allison et al., 1992), the two athlete groups do not have higher than normal dietary restraint scores.

Athletes participating in an aesthetic sport have been reported to have the highest prevalence of eating disorders due to the emphasis placed on leanness (Sundgot-Borgen, 1993). However, the literature in this area is controversial as it has been shown by some researchers that despite low energy intakes and excessive leanness, gymnasts have fewer tendencies towards eating disorders than other female athletes (Benson et al., 1990, Theintz et al., 1989). Figure skaters, who comprised approximately half the athlete population of this study are reported to have a high prevalence of eating disorders (Rucinski, 1989). Thus, if there were athletes with eating disorder tendencies in the study, the results of the dietary restraint questionnaire and energy intakes may be underestimated. Dietary restraint scores may be underestimated among the non-athlete controls as well because of the sociocultural expectation placed on all females to be thin, with adolescence being a time of particular vulnerability. Recruitment bias of the non-athlete controls may also have caused an underestimation of the dietary restraint scores and energy intake values if the controls were interested in weight control and energy balance. The Dutch Eating Behaviour Questionnaire provides valuable information about restrictive eating patterns, however it should not be used as a tool for determining the presence of an eating disorder.

2. Energy Expenditure

Total energy expenditure among the three groups was not significantly different. Activity calories were the only component of TEE that was different among the three groups, and both athlete groups expended significantly more calories than the non-athlete controls. Absolute REE was similar for the three groups, however, the oligo/amenorrheic athletes had a significantly greater REE relative to body weight (REE/kg) compared with the non-athlete controls with no difference between the two athlete groups. Because TEF was predicted from REE and not measured, it cannot be established whether differences existed for TEF among the three groups. Predicted TEF was used to estimate the extra calories required for the digestion of food to be used in the determination of TEE. Therefore, it can not be ascertained whether TEF was reduced and played a role in the conservation of energy expenditure.

3. Energy Balance

Although not significant, the oligo/amenorrheic were in a greater negative energy balance than the eumenorrheic athletes. The differences in energy balance between the three groups were not significant and this is most likely due to the variation in the energy intake data, which is expected considering the age range of the subjects. If this data was extrapolated to a larger sample size over a period of weeks to months, it is speculated that the energy balance trends between the three groups would become significant.

4. Evidence of Energy Conservation

It has been suggested that a negative energy balance as a result of chronic dieting or restrained eating with or without exercise may play a role in energy conservation by stimulating the body to lower REE and increase food/energy efficiency (Myerson et al., 1991, Brownell et al., 1987). REE for the three groups was not significantly different and there does not appear to be any evidence of energy conservation (REE was approximately 100% that of predicted REE for all groups). When adjusted for body weight, the oligo/amenorrheic athletes had a significantly greater REE compared to the non-athlete controls. There were no differences between

the two athlete groups or between the eumenorrheic athletes and the non-athlete controls. Sum of skinfolds and relative REE were negatively correlated suggesting that the greater relative REE of the oligo/amenorrheic athletes may be because they possessed a greater amount of LBM than the non-athlete controls. This is not a surprising finding since a greater amount of LBM has been found to increase REE (Whitney, Hamilton, Rolfes, 1990).

Another mechanism hypothesized to be an energy conserving mechanism for athletes in a negative energy balance is that they may be conserving energy by compensating for their high levels of physical training by reducing activity outside of training (Barr, 1987). However, it can not be determined from this study whether the oligo/amenorrheic athletes who were in the greatest negative energy balance were compensating by lowering their activity outside of training because this was measured.

Progesterone has thermogenic properties and is associated with an increase in REE (Webb, 1986, Bisdee & James, 1983). Energy intake has been reported to increase significantly during the luteal phase (Dalvit, 1981) which coincides with an increase in REE, so that energy balance is maintained (Barr, 1987). A reduction in REE was not reported for the oligo/amenorrheic athletes with low luteal phase progesterone levels. It is hypothesized that suppressed luteal phase progesterone levels may be another adaptive mechanism to conserve energy during negative energy balance (Webb, 1986). This hypothesis was not adequately tested in this study because luteal phase progesterone levels were measured while REE was determined during the follicular phase. Thus, it is possible to assume that the oligo/amenorrheic athletes may have been conserving energy during the luteal phase of the menstrual cycle or not compensating for the increase in REE by consuming extra calories. REE during the luteal phase (post-ovulation) has been reported to increase by 8 to 15% (Webb, 1986, Bisdee & James, 1983). An 8 to 15% increase in REE would increase the REE of the oligo/amenorrheic athletes by approximately 117 to 219 kcal respectively. If the increase in REE was not compensated for by an increased energy intake, theoretically a negative energy balance of 407 to 509 kcal would result.

Amenorrheic distance runners have been reported as maintaining stable body weights despite insufficient energy intakes needed to compensate for their high energy

expenditure levels (Myerson et al., 1991, Deuster et al., 1986b). It therefore appears that maintenance of stable body weights has a physiological priority over normal menstrual function. Barr (1987), suggests that by maintaining lower than normal body weight, energy requirements may be reduced.

It is speculated that the negative energy balance present in the oligo/amenorrheic athletes was not great enough to manifest symptoms of energy conservation. It is possible that the delayed pubertal development, low fat mass and body weight and the lower luteal phase progesterone levels among the oligo/amenorrheic athletes were a means to conserve energy when faced with a chronic mild negative energy balance. A reduction in REE may be the last stage of energy conservation that takes place, or it may occur only in states of severe negative energy balance. Fogelholm et al., (1995) did not find evidence of a reduction in REE despite a negative energy balance among female gymnasts and figure skaters in comparison to non-aesthetic athletes (soccer players), and non-athlete controls. However, the study did not control for the phase of the menstrual cycle during the measurement of REE, which may lead to an overestimated REE if measured during the luteal phase. To date, the only studies which have found evidence of an energy conservation have been those that investigated female distance runners and females with anorexia nervosa.

V. Menstrual Cycle Characteristics and Progesterone Analysis

Amenorrhea is common among aesthetic athletes. Of the ten oligo/amenorrheic athletes, four were classified as amenorrheic. Of the amenorrheic athletes, three were gymnasts who had only ever had one menstrual cycle. One gymnast was 16 years of age who had her first and only cycle at age 16. Another gymnast, 15 years of age, reached menarche when she was 15 and had not had another cycle since. Another gymnast, also 15 years old, reached menarche when she was 13 and was amenorrheic. It is common for menarche to be characterized by irregular and anovulatory cycles as the hypothalamic-pituitary axis is still immature. One synchronized swimmer with a gynecological age of 5 was amenorrheic, with a cycle length of approximately 100 days. Refer to Appendix XIV for individual data on menstrual cycle length and progesterone profile.

A late menarcheal age is characteristic of an immature reproductive axis which predisposes an athlete to menstrual irregularities (Loucks & Horvath, 1985). The finding of a younger gynecological age among the oligo/amenorrheic athletes may correspond with the greater prevalence of menstrual irregularities and longer mean cycle lengths. The age of menarche is hypothesized to correlate with the onset of ovulatory cycles; the earlier age of menarche, the earlier ovulation or ovulatory cycles occur (Vuorento & Huhtaneimi, 1992). In partial agreement to this study, Vuorento and Huhtaneimi (1992) found that females with a gynecological age of 1 to 2 years had significantly longer mean cycle lengths than the females with older gynecological ages.

It is difficult to determine menstrual dysfunction on the basis of menstruation frequency alone. For example, normal total cycle lengths typically characterize luteal phase deficiency. Bullen et al., (1985) found that hormone assessment increased the incidence of abnormal cycles from 60% to 89% in 53 cycles for 32 women. Therefore, the assessment of luteal phase progesterone levels should give a better indication of incidence of menstrual dysfunction, specifically corpus luteum function. Low luteal phase progesterone concentrations are a characteristic finding among elite athletes who endure chronic training. The oligo/amenorrheic athletes had significantly lower mean luteal phase progesterone concentrations compared to both the eumenorrheic athlete and the non-athlete controls. Whereas the mean luteal phase progesterone concentration of the eumenorrheic athletes and the non-athlete controls were similar. Differences in progesterone concentrations between the oligo/amenorrheic and the eumenorrheic athletes may be attributable to the inclusion of four amenorrheic athletes in the oligo/amenorrheic group. Individuals with amenorrhea display abnormal reproductive hormone profiles, with no phasic elevations in estrogen or progesterone. Such hormonal patterns are evidence of anovulation with no follicular and luteal development (Loucks, 1990).

There was a high frequency of athletes in the oligo/amenorrheic group who had only had their menarcheal cycle and were amenorrheic. Nevertheless, all but one reached normal luteal phase salivary progesterone concentrations of 100 to 200 pg/ml (0.1 to 0.2 ng/ml), indicative of ovulation. This finding is surprising since ninety percent of menstrual cycles in the first year of menarche are anovulatory (Goldfein &

Monroe, 1997). However, there is controversy concerning normal salivary luteal phase progesterone levels. Choe et al., (1983) and Luisi et al., (1981) have reported luteal phase levels which are higher than the consensus. Therefore, the incidence of anovulatory cycles in this study may have been underestimated or not captured in the analysis of a single menstrual cycle.

Reduced luteal phase progesterone levels are often indicative of corpus luteum defects as a result of imbalances of LH and FSH, with inadequate follicular development during the follicular phase (Strott et al., 1970, Sherman & Korenman, 1974, Soules et al., 1984). Low body weight, fat mass, dietary restraint, a negative energy balance and intensive exercise regimes have been linked to low luteal phase progesterone levels. The two athletes groups differed primarily only in menstrual cycle status and progesterone concentration with no variables correlated with progesterone levels. Thus the variables directly related to physical training do not appear to be related in the etiology of menstrual dysfunction among this population.

A low percentage of body fat and low body weights have been linked with menstrual abnormalities (Frisch and McArthur, 1974). Although not significant, body weight and fat mass were slightly lower among the oligo/amenorrheic athletes compared to the eumenorrheic athletes. It has been suggested that the rate of weight or fat loss plays more of an important role in menstrual dysfunction, however, these variables were not monitored. Other variables implicated in menstrual dysfunction that were not investigated in this study include the mental stress of physical training and the incidence of eating disorders. Although the dietary restraint score may have given some indication of eating disorder tendencies, the Dutch Eating Behaviour questionnaire does not determine the presence of eating disorders.

The low luteal phase progesterone levels may be related to the less developed maturation of the oligo/amenorrheic athletes. Even though the gynecological age and the puberty index were not significantly different among the two athlete groups, the difference of a year and a maturation stage respectively, may cause a clinical significant difference for progesterone levels and menstrual cycle status. In partial agreement with this theory, Vuorento and Huhtaneimi (1992) found that adolescent females with a gynecological age of 1 to 2 years had significantly longer mean cycle lengths than those

of an older gynecological age. However, luteal phase progesterone concentrations were lowest among the females with a gynecological age of 3 to 4 years. The results of this study suggest that when a female first becomes ovulatory, the luteal phase progesterone secretion is immediately precise and comparable to fertile adult women. However, after a couple of years, there is unknown disturbance of luteal phase progesterone secretion. Then about five or six years after menarche, the luteal phase is again comparable to fertile adult controls. This suggests that corpus luteum function, measured by progesterone production does not improve with advancing gynecological age. Whereas, Metcalf et al., (1982) reported incidence of ovulation to increase with gynecological age, with the highest incidence of ovulatory cycles reported in females with a gynecological age of greater than 5 years.

It may be speculated that although not significantly different, the subtle differences in body weight, fat mass, dietary restraint, negative energy balance, gynecological age and puberty index may be enough to cause the lower luteal phase progesterone levels among the oligo/amenorrheic athletes. The combination of the lower body weight, fat mass, gynecological age and pubertal maturation, the higher dietary restraint scores and negative energy balance may have had an impact on the luteal phase progesterone levels of the oligo/amenorrheic athletes. Because the incidence of eating disorders was not directly assessed, the oligo/amenorrheic athletes may have had more eating disorder tendencies than the other groups. This may have resulted in an underestimation of the dietary restraint score, energy intake and the negative energy balance.

Intense physical training has been reported to cause hormone alterations, which are implicated in the etiology of menstrual dysfunction (Bullen et al., 1985, Warren, 1980, Bonen et al., 1981, Jurkowski et al., 1978). Therefore, it was hypothesized that the two athlete groups would have lower progesterone levels than the non-athletes as a result of their intensive training regimes. The finding of lower progesterone concentrations among the oligo/amenorrehic athletes was expected. However, the similar progesterone concentration of the eumenorrheic athletes and the non-athlete controls is not in accordance with the hypothesis. Based on the results of this study and the variables measured, this finding cannot be adequately defined. However, the lower

than expected progesterone concentrations among the non-athletes is most likely related to a still immature hypothalamic-pituitary-ovarian axis which is characteristic of adolescence irrespective of training. This finding may also be attributed to a bias in subject selection since only females with an interest in participating were recruited who may also have had some subtle menstrual abnormalities that were not revealed by the data. The non-athlete controls may have joined the study because they were interested in and struggled with weight control, which may have underestimated the incidence of restrained eating and even eating disorder tendencies. This would have underestimated the theoretical reference value of normal progesterone concentrations from which the two athlete groups are compared to.

Another possible explanation may be because the non-athlete controls were significantly heavier and had a sum of skinfolds (between the 5^{th} and 10^{th} percentile) that was associated with estimated health risk zones according to trends in morbidity and mortality for their age and gender group (CSTF, 1986). The sum of skinfolds value classified the non-athlete controls as having an increased risk of health related problems related to obesity, whereas Health Canada (1980) height for weight percentiles classified this group as being at a good weight for their height. Excessive weight may also cause hormone alterations implicated in the etiology of menstrual dysfunction (Shoupe, 1991). Thus, it is reasonable to suspect that menstrual dysfunction was present in some of the non-athlete controls that did not surface during the initial screening of menstrual cycle status.

The analysis of individual progesterone profiles suggests that there is large variability within each group, irrespective of sport, athletic training and diet. Adolescence is characterized by variable and unpredictable hormonal profiles, therefore, the variability within each group is most likely a result of the age range and maturity levels of the subjects. There are a fair number of menstrual cycles that exhibited dual progesterone peaks; one around day 12 to 14 and one around day 25. The higher than expected progesterone levels around day 12 to 14 of the cycle was not expected and it is not clear what this progesterone peak is representing. This early peak in progesterone may be due to the cross-reactivity of the assay with other metabolites of progesterone. The percent cross-reactivity of the assay with other progesterone

metabolites was as follows; 2.2% for 20α-dihyroprogesterone, 1.1% for 17α-hydroxyprogesterone and 4.8% for 5α-pregnan-3-20-dione. Therefore, complete hormonal analysis of adolescent menstrual cycles may warrant further investigation.

VI. Limitations of the Study

Limitations of the study include the challenges inherent in the measurement of energy intake and energy expenditure. It is because of these limitations that the energy balance data are only an estimate as it is based on one time point only, which may not be representative of usual energy balance status. Determining energy intake is difficult in the study of free living individuals. There are several reasons why absolute energy intake values may be unreliable. First, food recording frequently causes an underestimation of energy intake of both adults and children (Bandini et al., 1990, Edwards, 1993). Second, three-day food records represent a short period for assessing energy intake and may not reflect daily variability in intakes (Thompson, 1998, Rankinen et al., 1995). However, a longer recording period was regarded as impractical for this group because adolescents are prone to recording errors and the demanding training schedule of the athletes limited their time availability for the study. Lastly, there are problems in quantifying the amount of food eaten, as well as the type of food.

The measurement of total energy expenditure was also only an estimate and because no other study has determined TEE using the combination used in this study, makes it difficult to make comparisons. There are also the possible problems of using the TriTrac-R3D® to estimate physical activity calories. The TriTrac-R3D® and other triaxial accelerometers have been reported to underestimate physical activity calories compared to self-report activity diaries (Matthews & Freedson, 1995, Epstein et al.,1996). The TriTrac-R3D® was chosen over self-report activity diaries because it is a more objective measure of energy expenditure and although the TriTrac-R3D® may have resulted in an underestimation of energy expenditure and subsequently energy balance, the underestimation would have been uniform across all three groups. Several studies determining TEE in adults have added additional calories to account for "other" daily activities such as sitting, standing and fidgeting that are not recorded on the activity diaries. For example, Wilmore et al., (1992) multiplied REE by 1.1 and

Myerson et al., (1990) used 9% of REE to account for such activities. This study did not add additional calories for these activities because the accelerometer proposed to measure such movement. The reliability and precision of triaxial accelerometers is still being investigated. Therefore it is not known whether the activity from "other" activities was actually measured by the accelerometer and to what degree of accuracy.

Energy intake is reported to increase substantially during the luteal phase by approximately 500 kcal extra per day, most likely due to the thermogenic properties of progesterone (Dalvit, 1981). This study measured energy intake on one occasion only, for three days, between day one and ten of the follicular phase. Due to the considerable variation in energy intakes that coincides with the phase of the menstrual cycle, energy intake should be assessed during the follicular phase and during the luteal phase. This would reflect usual energy intakes over the menstrual cycle and determine whether the athletes were compensating for the increase in REE during the luteal phase by increasing energy intake. Correspondingly, REE should have also been measured during both the follicular phase and the luteal phase to determine whether the low luteal phase progesterone levels are associated with a reduced REE in the oligo/amenorrheic athletes. The REE measured during the luteal phase could then be compared to the REE measured during the follicular phase to determine whether individuals with low luteal phase progesterone levels are conserving energy during the luteal phase as opposed to the follicular phase.

Problems with the measurement and analysis of the progesterone data include the variability of subject criteria and the uncertainty of data alignment. The inclusion of 4 amenorrheic athletes in the oligo/amenorrheic athlete group may have skewed the results of the progesterone data since amenorrhea is characterized by low progesterone concentrations. Progesterone sampling was intended to reflect the secretion of progesterone over the luteal phase starting at Day 12 of the cycle. Most published standards for salivary progesterone use the day of the LH peak or ultrasound to detect ovulation. Therefore, it is difficult to determine whether a true luteal phase was captured for those subjects who were amenorrheic since a random day was chosen to represent Day12 for these subjects. The self-reporting of menstrual onset may have also resulted in inaccurate results.

Lastly, because adolescence is characterized by irregular menstrual cycle patterns and hormonal profiles, it is difficult to determine whether the menstrual cycle abnormalities and the low progesterone levels of the oligo/amenorrheic athletes were because of an immature hypothalamic-pituitary-ovarian axis or due to some aspect of training. For purposes of comparison, future research should place amenorrheic athletes in a category on their own and analyze the data separately from athletes who are oligomenorrheic and eumenorrheic.

VII. Conclusions and Recommendations for Future Research

Results from this study imply that aesthetic athletes have a later age of menarche and a slower maturation of the secondary sex characteristics in comparison to non-athletes. It may be speculated that these findings may be related to the effects of intense training prior to puberty, hereditary and self-selection of their sport. In order to assess the influence of athletic training upon these variables future research should assess the role of genetics by determining parental height and maternal age of menarche of the subjects.

Differences in progesterone levels between the two athlete groups cannot be solely attributable to physiological factors and intense physical training because there were no significant differences among the two athlete groups on any of these variables. The two athlete groups differed primarily only in gynecological age, with the oligo/amenorrheic athletes having a younger gynecological age than the eumenorrheic. Whereas, the eumenorrheic athletes and the non-athlete controls differed significantly in physiological factors, however, had similar gynecological age. Thus it is reasonable to assume that gynecological age is related to luteal phase progesterone levels and maintenance of regular cycles. The lower luteal phase progesterone levels may also be attributed to the subtle differences in physiological and lifestyle factors among the two athlete groups. Factors such as greater negative energy balance and restraint scores, slower maturation, a younger gynecological age, lower body weights and fat mass may act synergistically in contributing to the menstrual dysfunction present among the oligo/amenorrheic athletes. The lower luteal phase progesterone concentrations among the oligo/amenorrheic athletes was expected considering this group included four

amenorrheic athletes, who typically have very low luteal progesterone profiles. However, it is difficult to determine from the results of this study which factors were involved in the etiology of low progesterone levels since both athlete groups were similar.

Based on the result of the REE data and despite the inherent limitations of this study, there was no direct evidence of energy conservation for the oligo/amenorrheic athletes who were in the greatest negative energy balance. However, indirect signs of energy conservation were present among the oligo/amenorrheic athletes which included, delayed maturation, low luteal phase salivary progesterone levels, low body weights, and sum of skinfolds. For greater precision in the measurement of energy balance, future research should employ nitrogen balance and doubly labelled water techniques. Although costly and labour intensive, these methods are more accurate in assessing energy balance in children and adolescents than self-report methods. It is apparent that adolescent aesthetic athletes are particularly susceptible to the effects of a negative energy balance. Further investigation is therefore necessary to establish standards for the prevention, treatment and long-term implications of the negative health consequences of a negative energy balance.

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University of Alberta

Inter-departmental Correspondence
Faculty of Agriculture, Forestry, and Home Economics
Office of the Dean

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to: Karen Reading, BASc, AFNS
Linda McCargar, PhD, RD, AFNS
Vicki Harber, PhD, Phys. Ed. & Rec.

date: July 11, 1997

from: Dr. B. Christopherson
Associate Dean (Research)

our file christop\ethics\july11.97\mccargar

subject: Re: Activity, Diet and Menstrual Status in Adolescent Women: Athletes vs Non-athletes

your file:

The above proposal is approved as meeting the requirements of the Faculty policy on Human Ethics in Research. However, the following points are raised for your consideration:

1. Is the term *aesthetic athletes* a term that is widely understood?
2. Collection, handling and disposal of salivary samples likely require biosafety approval.
3. In the event that a participant develops anxiety with respect to menstrual health status, do you have a plan or mechanism for referral to an appropriate profession?

Your sincerely,

Dr. R. J. Christopherson

cc: P. Woodard, Associate Dean (Research)

RJC/acc
(christop\ethics\july 11\mccargar)

University of Alberta
Edmonton
Canada T6G 2P5

Department of Agricultural, Food and Nutritional Science
Faculty of Agriculture, Forestry, and Home Economics
410 Agriculture/Forestry Centre
Telephone: (403) 492-3239
Facsimile: (403) 492-4265
E-mail: chair@afns.ualberta.ca

Parental Consent Form

ACTIVITY, DIET AND MENSTRUAL STATUS IN ADOLESCENT WOMEN

	Yes	No
Do you understand that you have been asked to be in a research study?	[ ]	[ ]
Have you read and received a copy of the attached information sheet?	[ ]	[ ]
Do you understand the benefits and time requirements involved in this study?	[ ]	[ ]
Do you understand that you are free to withdraw from the research study at any time, without having to give to a reason and without prejudice?	[ ]	[ ]
Do you understand that if any knowledge is gained from the study that could influence your decision to continue in this study, you will be promptly informed?	[ ]	[ ]
Has the issue of confidentiality been explained to you and do you understand who will have access to your data?	[ ]	[ ]
I agree to take part in this research study?	[ ]	[ ]

If participant is under 18 years of age, signature of parent/legal guardian is required.

Name of participant

Signature of participant

Name of parent/legal guardian

Signature of parent/legal guardian

Name of Witness

Signature of Witness

Date

...it makes sense. UNIVERSITY OF ALBERTA

University of Alberta
Edmonton
Canada T6G 2P5

Department of Agricultural, Food and Nutritional Science
Faculty of Agriculture, Forestry, and Home Economics
410 Agriculture/Forestry Centre
Telephone: (403) 492-3239
Facsimile: (403) 492-4265
E-mail: chair@afns.ualberta.ca

INFORMATION SHEET

ACTIVITY, DIET AND MESNTRUAL STATUS IN ADOLESCENT WOMEN

INVESTIGATORS:

Karen Reading	MSc Candidate	492-4267
Linda M^{c}Cargar	PhD. RD.	492-9287
Vicki Harber	PhD.	492-1023

Background:
Menstrual irregularities are fairly common in females who participate in strenuous physical activity. Endurance and aesthetic athletes such as gymnasts, figure skaters and dancers are particularly at risk for menstrual irregularities. Previous studies have suggested that some females may have changes in their body metabolism, diet, activity level, body weight and/or body fat. Inadequate caloric intake has been suggested as one of the causes of menstrual irregularities, especially when in combination with intense physical training. Activity levels can now be easily measured and energy expenditure calculated using a technique that allows females to continue their usual levels of physical activity. The present study will measure activity and metabolism using a number of tests and questionnaires.

Purpose:
The purpose of the present study is to compare measures of physical activity, diet and metabolism in non-athlete adolescent females, adolescent female athletes with normal menstrual cycles and a similar group of adolescent female athletes with infrequent menstrual cycles.

Procedures:
Each participant will require three visits to the University of Alberta where a series of tests and questionnaires will be performed. The investigations are as follows:

Pen and Paper Questionnaires. A number of questionnaires related to general health (including fequency of menstrual cycles), diet and physical activity.
Body Composition: Height, weight, skinfold thickness at 5 sites will be measured.
Resting Metabolic Rate: The amount of energy used at rest will be measured by collected the gases from the lungs in a rested and fasted conditions. This procedure takes about 2 hours.
Diet Diaries: Each subject will keep a 3 day diary of food intake.
Activity Patterns: An activity diary will be kept for one week. The specific activity, duration and intensity of effort will be charted.

Activity Monitoring: Each subject will wear a device on a belt that measures movement for 3 days. The device does not interrupt activity in any way and involves no risk.
Saliva Samples: Each subject will be asked to produce salivary samples for 2 –3 weeks. This procedure is for the measurement of a hormone called progesterone. The procedure involves chewing on a cotton wad for approximately 1 minute in the morning before eating or brushing teeth. The cotton wad is then stored within a plastic tube-like container in a home freezer until the collection of samples is complete.
Aerobic Fitness Testing: Aerobic fitness will be determined by measuring maximal oxygen uptake (VO_2Max) during progressive exercise on a stationary bicycle until the subject is no longer able to continue. Subjects will breath through a mouthpiece and the oxygen and carbon dioxide will be measured as well as heart rate.

Possible Benefits:
Each individual will have access to her own information about her fitness, activity, diet, body composition and metabolism if she wishes. It is anticipated that the study will provide information about exercise intensity, aerobic fitness and nutritional status.

Possible Risks:
There should be no adverse effects to the subjects participating in this study. There are no risks to answering the questionnaires, wearing the activity monitor, body composition measurements or measuring resting metabolic rate. Aerobic fitness testing may induce muscle soreness, shortness of breath and temporary abnormal heart beat and blood pressure.

Confidentiality:
Personal records relating to this study will be kept confidential. Code numbers will be used rather than names and all files will be stored in a locked file cabinet. Any report published as a result of this study will present group results only and no individual will be identified.

Results:
Each participant will have the opportunity to review her results with the researcher. These results include body composition, salivary progesterone, fitness levels and diet adequacy.

Withdrawal From Study
You are free to withdraw from the study at any time without jeopardy. If any knowledge gained from this or any other study becomes available which could influence the decision to continue in the study you will be promptly informed. Under all circumstances, the information gathered from this study, relevant to each participant will be communicated to that participant in an open, yet confidential manner. It will be the participants decision to continue participation in all stages of this study.

Any Questions?
Please contact Karen Reading, MSc Candidate, Department of Agricultural, Food and Nutritional Science. Phone number: 492-4267.

MENSTRUAL HISTORY and HEALTH QUESTIONNAIRE

General Information

Name: _____ Date: _____

Telephone Number: _____

Date of Birth: _____ Age: _____

Have you experienced a weight loss or gain (6-8 pounds or more) in the last 12 months?
Yes _____ No _____

If yes, specify the amount of weight lost (-) or gained (+) _____

List any prescribed medications or over-the counter medications you are currently taking.

_____

_____

Do you have any chronic or "nagging" muscle musculoskeletal aches or pains (ie. sore knees, weak back)? Yes _____ No _____

If yes, indicate the location of your ache or pain and describe any related physical limitations.

_____

_____

Menstrual Cycle Characteristics

At what age did you have your first menstrual period?
(Please answer to the nearest month) _____

Have you taken oral contraceptive pills within the last 6 months?

Yes _____ No _____

Is your menstrual cycle regular. (ie. about every 25-35 days)?

No_____ When was the last time you menstruated? _____

How many periods do you usually have in a year? _____

What is the longest time you have gone without a period? _____

Yes ____ How many periods do you usually have in a year? _____

What is the interval of days between your periods (ie. the number of days between day 1 (first day of flow) and day 1 of the next cycle)?

_____

On average, how many days does your period last? _____

What is the date of your last menstrual period? _____

Physical Activity

Are you currently involved in a regular routine of physical activity or exercise? (Minimum of 3 times per week for the last 4 consecutive months)

No ——— (If no, go to the last question on this page)
Yes ——— (If yes, answer the next 7 questions)

What sport are you currently training in? _____

How many years have you been involved in this sport? _____

At what age did you start competing? _____

How many days per week do you train? _____

How many hours per week do you train? _____

Please fill in how many hours each day you train.

Sun	Mon	Tue	Wed	Thur	Fri	Sat
____	____	____	____	____	____	____

Is this your usual schedule? _____ If not, how long has this been your training schedule? _____

Please feel free to add other comments you think are important for us to know:

_____

Appendix V. The Tanner Visual Pubertal Self-Assessment Scale

A Non-invasive Method for Determining Maturational Status in Children and Adolescents

Maturity Index

Subjects Initials:	Birthdate:
Subject Code:	Date:

Instructions:

NOTE: The following introduction (written or/and verbally) is given to each child prior to the assessment.

"As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children, and you may already be seeing some changes, other may have already gone through some changes. Sometimes it is important to know how a person is growing without having a doctor examining them. It can be hard for a person to describe herself or himself in words, so doctors have drawings of stages that all children go through. There are 5 drawings of pubic hair growth which are attached for you to look at.

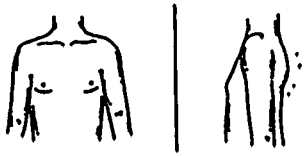
I would like to know how well you can select your stage of growth from the set of drawings. All you need to do is pick the drawing that looks like you do now. Put a check mark above the drawing that is closest to your stage of development then put the sheet in the envelope and seal it so your answer will be kept private".

The drawings on this page show <u>different stages of female breast development.</u> Please look at each of the drawings and read the sentences beside the drawings. Then check the drawing that is <u>closest</u> to your stage of breast development.

Picture 1 _____

There is no breast development at all.

Picture 2 _____

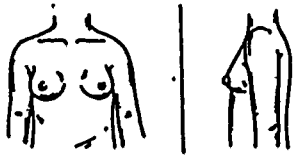


There is a small amount of breast budding. The diameter of the areola (area around the nipple) is increased.

Picture 3 _____

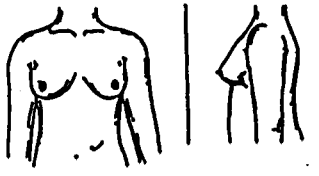
The breast and areola are raised and enlarged.

Picture 4 _____



The areola and the nipple are raised and separate from the rest of the breast.

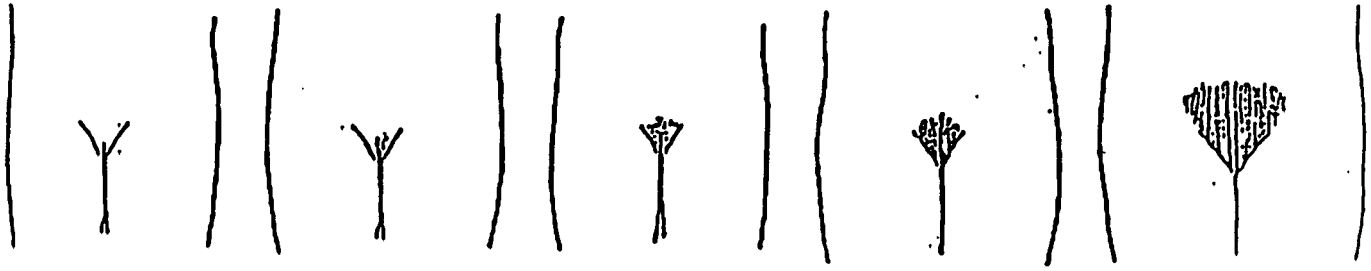
Picture 5 _____



The areola has flattened to the level of the breast and only the nipple is raised.

The drawings on this page show <u>different amounts of female pubic hair</u>.
Please look at each of the drawings and read the sentences under the drawings.
Then check the drawing that is <u>closest</u> to your stage of hair development.

Picture 1 ___ Picture 2 ___ Picture 3 ___ Picture 4 ___ Picture 5 ___



There is no pubic hair at all.

There is a small amount of long, lightly colored hair. This hair may be straight or a little curly

There is hair that is darker, curlier and thinly spread out to cover a somewhat larger area than in stage 2.

The hair is thicker and more spread out, covering a larger area than in stage 3.

The hair now is widely spread covering a large area, like that of an adult female.

Physical Activity Readiness Questionnaire - PAR-Q (revised 1994)

PAR - Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2. Do you feel pain in your chest when you do physical activity?
☐	☐	3. In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7. Do you know of any other reason why you should not do physical activity?

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- If you are or may be pregnant — talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

You are encouraged to copy the PAR-Q but only if you use the entire form

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Supported by: Health Canada Santé Canada

Instructions:
Read each question slowly. Choose the statement that best applies to you. There are no right or wrong answers - this is not a test. Please answer all the questions as honestly and accurately as you can – this is very important.

Very Often	Often	Sometimes	Seldom	Never	
( )	( )	( )	( )	( )	1. When you have put on weight, do you eat less than you usually do?
			Not Relevant ( )		
( )	( )	( )	( )	( )	2. Do you try to eat less at meal times than you would like to eat?
( )	( )	( )	( )	( )	3. How often do you refuse food or drink offered because you are concerned about your weight?
( )	( )	( )	( )	( )	4. Do you watch exactly what you eat?
( )	( )	( )	( )	( )	5. Do you deliberately eat foods that are slimming?
( )	( )	( )	( )	( )	6. When you have eaten too much, do you eat less than usual the following day?
			Not Relevant ( )		
( )	( )	( )	( )	( )	7. Do you deliberately eat less in order not to become heavier?
( )	( )	( )	( )	( )	8. How often do you try not to eat between meals because you are watching your weight?
( )	( )	( )	( )	( )	9. How often in the evening do you try not to eat because you are watching your weight?
( )	( )	( )	( )	( )	10. Do you take into account your weight with what you eat?

MENU ITEM	UNIT OF MEAS.	No. of Units	DESCRIPTION OF MENU ITEM		
Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating	Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"		Brand	Type of Flavour	Method of Cooking
MORNING MEAL — Menu Item					
Toppings or Additives					
Menu Item					
Toppings or Additives					
Menu Item					
Toppings or Additives					
Menu Item					
Toppings or Additives					
Menu Item					
Toppings or Additives					
Menu Item					
Toppings or Additives					
Mark (X) One Category	Eaten at Your Home				
	Eaten Away From Your Home				
	Did Not Eat				

Day Two

NAME: _____ PHYSICAL ACTIVITY JOURNAL WEEK OF: _____

	Sunday		Monday		Tuesday		Wednesday		Thursday		Friday		Saturday	
Activity	Duration (Min)	Intensity (Circle #)	Duration (Min)	Intensity (Circle #)	Duration (Min)	Intensity (Circle #)	Duration (Min)	Intensity (Circle #)	Duration (Min)	Intensity (Circle #)	Duration (Min)	Intensity (Circle #)	Duration (Min)	Intensity (Circle #)
1.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5
2.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5
3.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5
4.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5
5.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5
6.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5
7.		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5		1 2 3 4 5

Intensity: 1 = Not vigorous at all (Very Light)
2 = Somewhat vigorous (Light)
3 = Moderately vigorous (Medium)
4 = Vigorous (Heavy)
5 = Extremely Vigorous (Very very heavy)

Appendix X. Sample calculations: Determination of activity calories from activity diaries

Sample Calculations: Determination of Activity Calories from Activity Diaries (METs)

Day 1

Swimming intensely for 15 minutes: MET value = 8.0

15 min/60 min = 0.25[8 x body weight (kg)]
= 0.25(8 x 67.9)
= 0.25(543.2)
= 136 kcal

Swimming (synchronized swimming) moderately for 35 minutes: MET value = 6.0

35 min/60 min = 0.58[6 x body weight (kg)]
= 0.58(6 x 67.9)
= 0.58(407.4)
= 236.3 kcal

Activity calories derived from water training for Day 1 = 372.3 kcal

Total activity calories for Day 1 (land training and water training):

= activity kcal from TriTrac-R3D® + activity kcal (activity diaries)
= 382.51 kcal + 372.3 kcal
= 754.81 kcal

ID bas3
Name
Time Stamp 25-Feb-98 10:48
Age 16
Gender F
Height (cm) 157
Weight (kg) 048
Minutes per Interval 01
Comment 1
Comment 2
Initial Battery Reading 137 8.6Volts
Current Battery Reading 134 8.4Volts
Initial Lithium Reading 154 3+ Volts
Current Lithium Reading 161 3+ Volts
Error Flag N
Power Fail N
PDU Serial Number 00872
PDU Version Number 03.00

Activity Calories = 1305.66

Number of points: 4358

Metabolic Calories per interval: 1.01
Total Metabolic Calories: 4401.58

Date & Time	RawCntX	RawCntY	RawCntZ	Vec.Mag	Act.Cals	Tot.Cals
25-Feb-98 10:48	218	115	249	350	0.62	1.63
25-Feb-98 10:49	149	562	394	702	1.25	2.26
25-Feb-98 10:50	224	446	352	610	1.09	2.10
25-Feb-98 10:51	494	957	896	1400	2.50	3.51
25-Feb-98 10:52	489	1138	838	1495	2.66	3.67
25-Feb-98 10:53	100	167	321	375	0.66	1.67
25-Feb-98 10:54	34	243	58	252	0.44	1.45
25-Feb-98 10:55	186	423	377	596	1.05	2.06
25-Feb-98 10:56	261	366	539	701	1.25	2.26
25-Feb-98 10:57	303	295	346	546	0.96	1.97
25-Feb-98 10:58	123	294	313	446	0.78	1.79
25-Feb-98 10:59	457	806	885	1281	2.29	3.30
25-Feb-98 11:00	382	860	783	1224	2.18	3.19
25-Feb-98 11:01	288	668	493	878	1.55	2.56
25-Feb-98 11:02	121	244	173	322	0.57	1.58
25-Feb-98 11:03	567	930	593	1240	2.21	3.22
25-Feb-98 11:04	307	454	349	649	1.14	2.15
25-Feb-98 11:05	54	127	55	148	0.25	1.26
25-Feb-98 11:06	28	33	108	116	0.19	1.20
25-Feb-98 11:07	111	93	107	180	0.32	1.33
25-Feb-98 11:08	55	67	50	100	0.17	1.18
25-Feb-98 11:09	93	100	106	172	0.30	1.31
25-Feb-98 11:10	370	715	512	954	1.70	2.71
25-Feb-98 11:11	64	70	115	149	0.25	1.26
25-Feb-98 11:12	29	18	25	42	0.07	1.08
25-Feb-98 11:13	65	107	124	176	0.30	1.31
25-Feb-98 11:14	64	74	105	143	0.25	1.26

Sample Size Calculations

Sample size (n) $= [(Z_\alpha + Z_\beta)^2\ 2(\sigma^2)]/D^2$

σ = standard deviation of response variable
D = want to detect difference of 2 standard deviations from the mean
Z_α = Type I error at 0.05 level of significance
Z_β = At 95% confidence the probability of declaring means as different

1.) Subjects: female adolescents with ovulatory cycles and 1 to 2 years gynecological age (n = 15)

Reference: Vourento and Huhtaniemi, 1992

Units of Measurement: log C95 = log of the cumulative sum of the total progesterone secreted in the luteal phase
$= 3.26 \pm 0.07$

$$n = \frac{[(1.96 + 1.64)^2\ 2(0.07)^2]}{(0.07)^2} \qquad n = 13 \text{ subjects}$$

2.) Subjects: female adolescents with ovulatory cycles and 3 to 4 years gynecological age (n = 29)

Reference: Vuorento and Huhtaniemi, 1992

Units of Measurement: log C95 = 0.05

$$n = \frac{[(1.96 + 1.64)^2\ 2(0.05)^2]}{(0.05)^2} \qquad n = 13 \text{ subjects}$$

Subjects: fertile female adults with ovulatory cycles

Reference: Choe et al., (1983)

Units of Measurement: average luteal phase progesterone concentration = 0.0398 (pg/ml)

$$n = \frac{[(1.96 + 1.664)^2\ 2(0.0398)^2]}{(0.0398)^2} \qquad n = 13 \text{ subjects}$$

K Reading, Re: copyright permission (fwd)

To: K Reading <kreading@gpu.srv.ualberta.ca>
From: Geoff Ball <gdball@gpu.srv.ualberta.ca>
Subject: Re: copyright permission (fwd)
Cc:
Bcc:

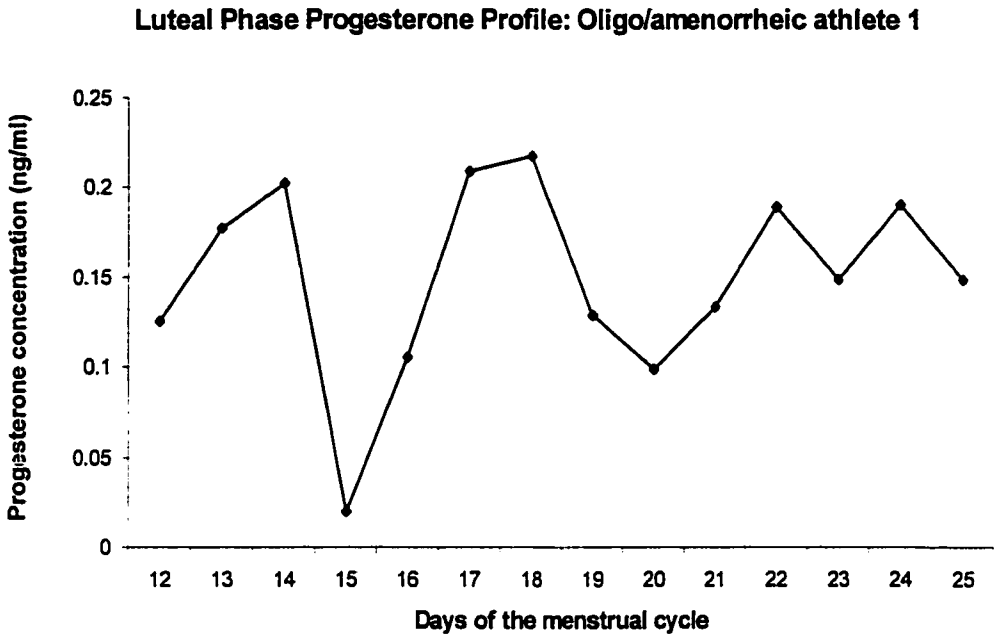
Date: Tue, 15 Sep 1998 08:59:35 -0400
From: "Robert J. Huskey" <rjh9u@blue.unix.virginia.edu>
To: K Reading <kreading@gpu.srv.ualberta.ca>
Subject: Re: copyright permission

Ms. Reading,

You have my permission to use the images you mentioned. Neither diagram is taken directly from a text; both are adaptations from several sources.

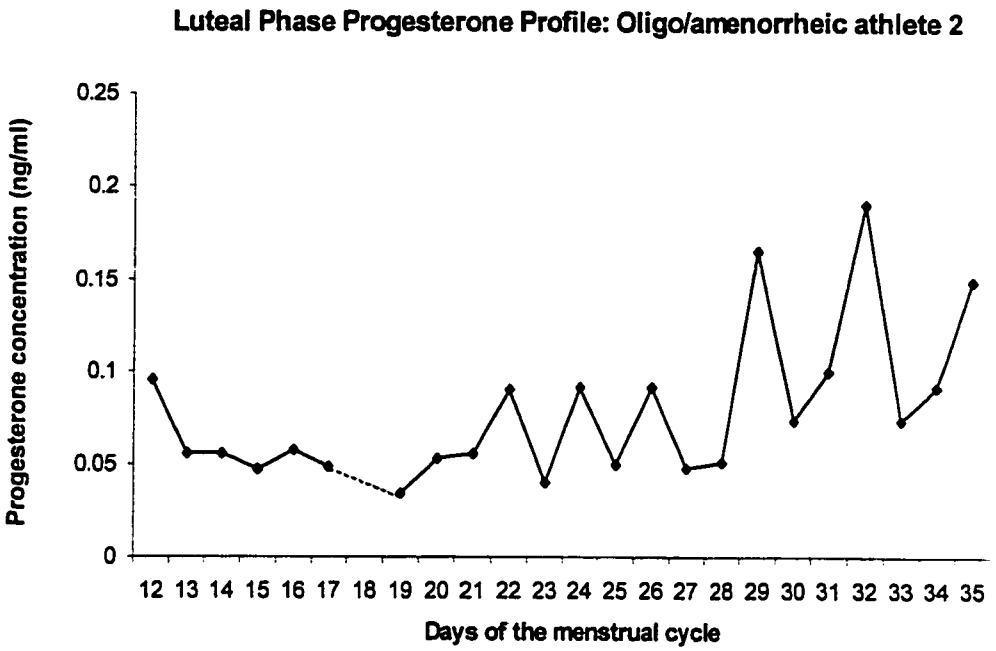
RJH

****
Robert J. Huskey
Associate Dean, Graduate Arts & Sciences
Associate Professor, Dept. of Biology
438 Cabell Hall, University of Virginia
Charlottesville VA 22903 (804) 924-7183
http://www.people.virginia.edu/~rjh9u/humbiol.html
****



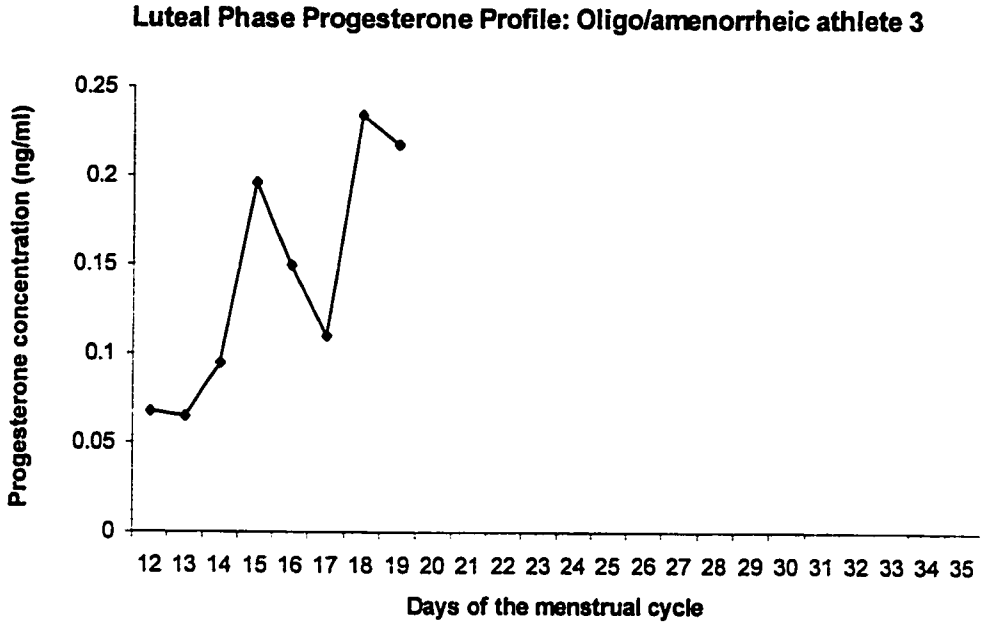
Individual Data for Oligo/amenorrheic Athlete 1

Athlete	Gymnast
Chronological Age (years)	15
Gynecological Age (years)	1
Menstrual Cycle Status	Oligomenorrheic
Mean Progesterone Concentration	0.149 ng/ml
Peak Progesterone Concentration	0.217 ng/ml
Body Weight	44.1 kg
Height	152 cm
Sum of 5 Skinfolds	53 mm
Dietary Restraint Score	2.7
Mean Energy Intake	1653 kcal/day
Mean Energy Intake/kg	37.5 kcal/kg/day
% Calories from Carbohydrates	64%
% Calories from Protein	10%
% Calories from Fat	27%
REE	1270 kcal/day
Mean Activity Calories	428 kcal/day
Mean TEE	1787 kcal/day
Energy Balance	- 134 kcal/day



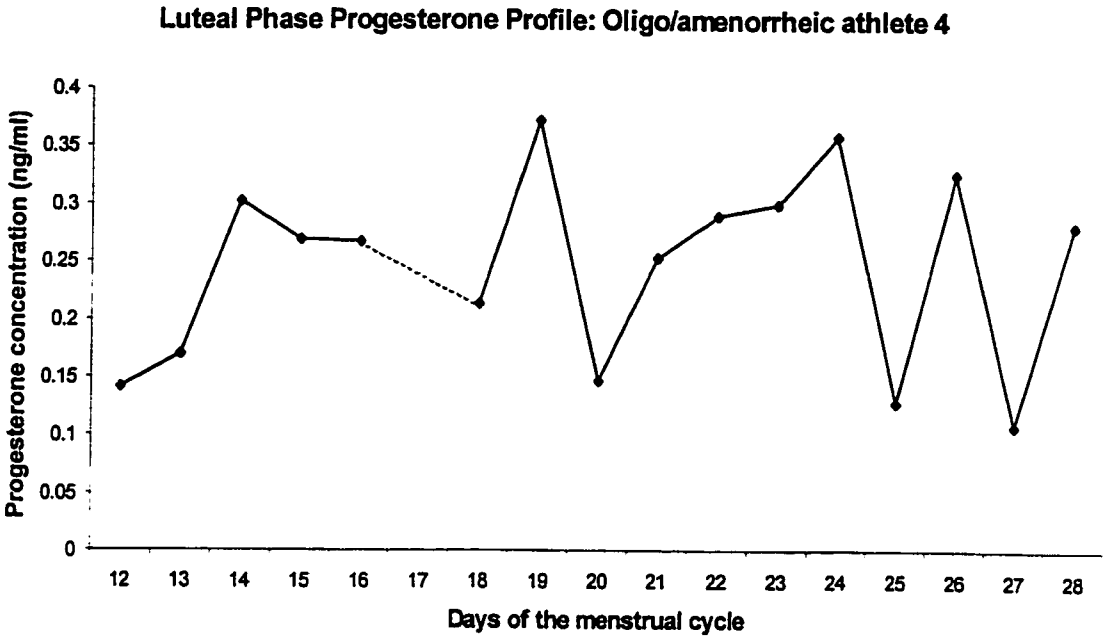
Individual Data for Oligo/amenorrheic Athlete 2

Athlete	Figure Skater
Chronological Age (years)	16
Gynecological Age (years)	3
Menstrual Cycle Status	Oligomenorrheic
Mean Progesterone Concentration	0.077 ng/ml
Peak Progesterone Concentration	0.191 ng/ml
Body Weight	66 kg
Height	169 cm
Sum of 5 Skinfolds	64.3 mm
Dietary Restraint Score	3.5
Mean Energy Intake	2448 kcal/day
Mean Energy Intake/kg	37.1 kcal/kg/day
% Calories from Carbohydrates	64%
% Calories from Protein	15%
% Calories from Fat	21%
REE	1530 kcal/day
Mean Activity Calories	523 kcal/day
Mean TEE	2160 kcal/day
Energy Balance	288 kcal/day



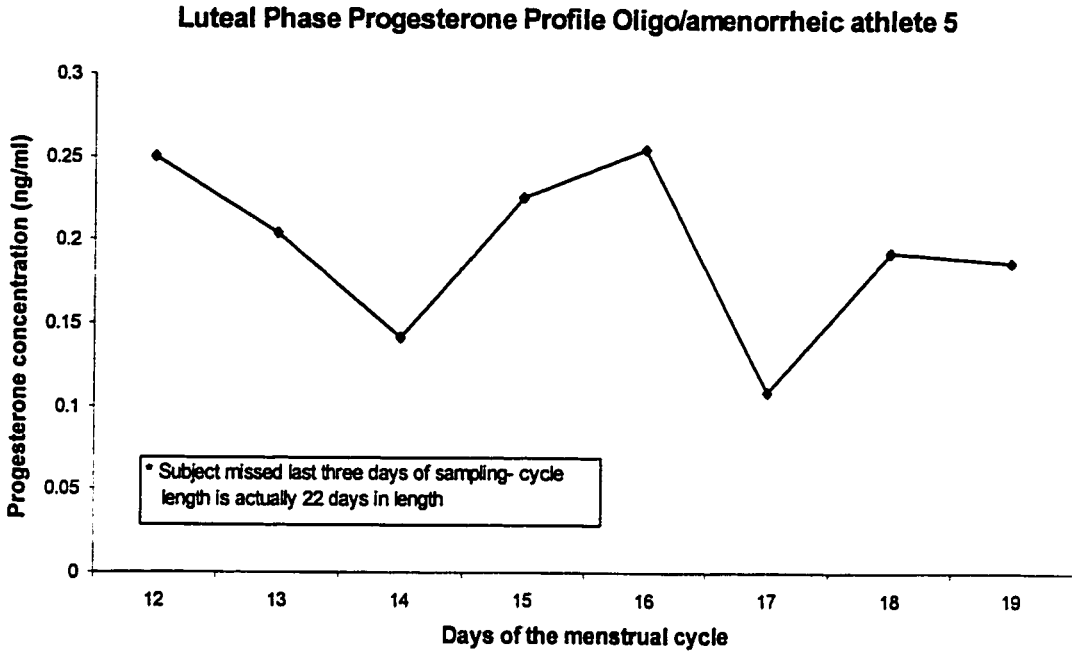
Individual Data for Oligo/amenorrheic Athlete 3

Athlete	Synchronized Swimmer
Chronological Age (years)	17
Gynecological Age (years)	5
Menstrual Cycle Status	Amenorrheic
Mean Progesterone Concentration	0.142 ng/ml
Peak Progesterone Concentration	0.234 ng/ml
Body Weight	52.4 kg
Height	165 cm
Sum of 5 Skinfolds	37.5 mm
Dietary Restraint Score	4.5
Mean Energy Intake	1174 kcal/day
Mean Energy Intake/kg	22.4 kcal/kg/day
% Calories from Carbohydrates	51%
% Calories from Protein	14%
% Calories from Fat	42%
REE	1352 kcal/day
Mean Activity Calories	873 kcal/day
Mean TEE	2319 kcal/day
Energy Balance	- 1145 kcal/day



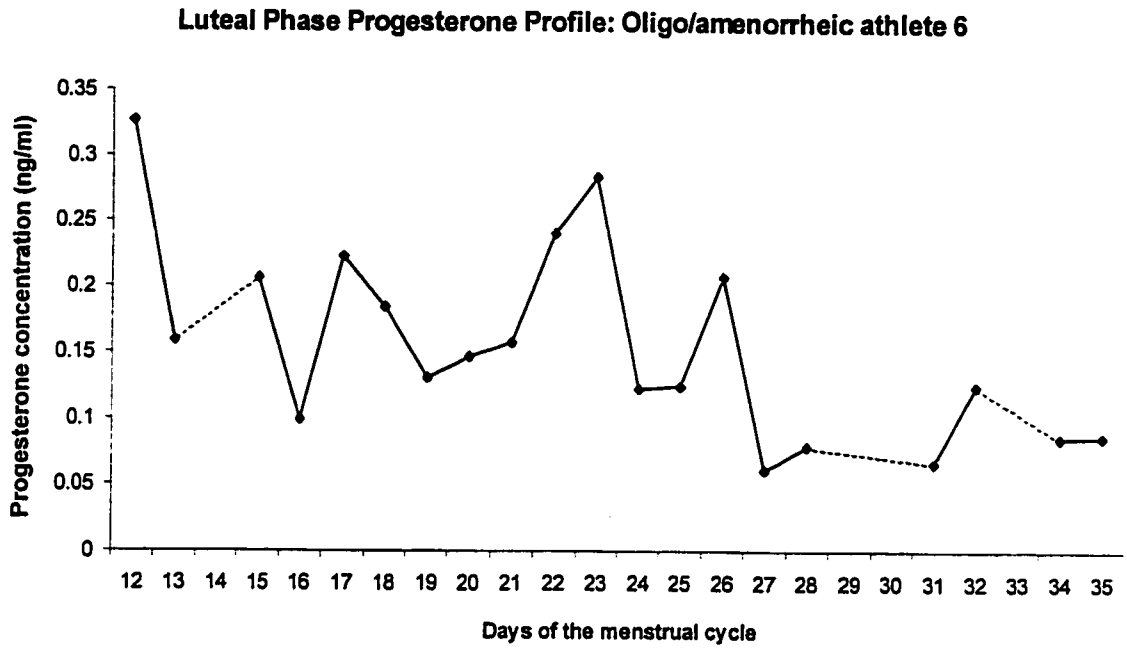
Individual Data for Oligo/amenorrheic Athlete 4

Athlete	Gymnast
Chronological Age (years)	15
Gynecological Age (years)	2
Menstrual Cycle Status	Amenorrheic
Mean Progesterone Concentration	0.245 ng/ml
Peak Progesterone Concentration	0.372 ng/ml
Body Weight	58.3 kg
Height	159 cm
Sum of 5 Skinfolds	54.5 mm
Dietary Restraint Score	3.1
Mean Energy Intake	1672 kcal/day
Mean Energy Intake/kg	28.7 kcal/kg/day
% Calories from Carbohydrates	62%
% Calories from Protein	12%
% Calories from Fat	26%
REE	1475 kcal/day
Mean Activity Calories	542 kcal/day
Mean TEE	2120 kcal/day
Energy Balance	- 448 kcal/day



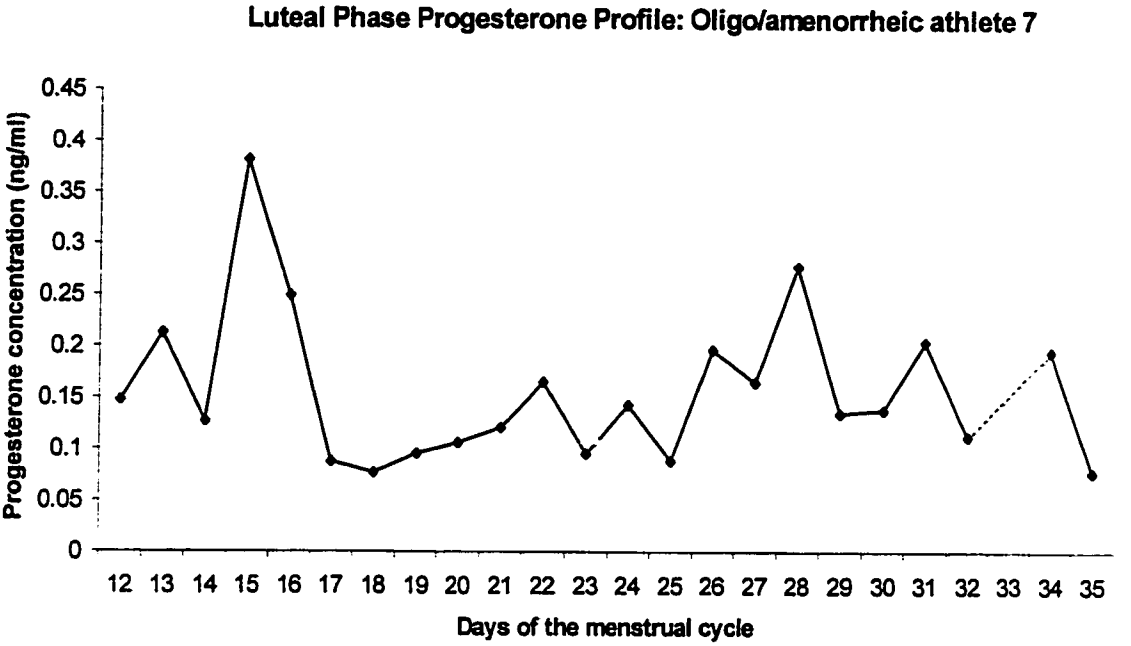
Individual Data for Oligo/amenorrheic Athlete 5

Athlete	Figure Skater
Chronological Age (years)	16
Gynecological Age (years)	3
Menstrual Cycle Status	Oligomenorrheic
Mean Progesterone Concentration	0.201 ng/ml
Peak Progesterone Concentration	0.255 ng/ml
Body Weight	74.2 kg
Height	165 cm
Sum of 5 Skinfolds	82.7 mm
Dietary Restraint Score	2.5
Mean Energy Intake	1205 kcal/day
Mean Energy Intake/kg	16.2 kcal/kg/day
% Calories from Carbohydrates	76%
% Calories from Protein	10%
% Calories from Fat	14%
REE	1714 kcal/day
Mean Activity Calories	981 kcal/day
Mean TEE	2815 kcal/day
Energy Balance	- 1610 kcal/day



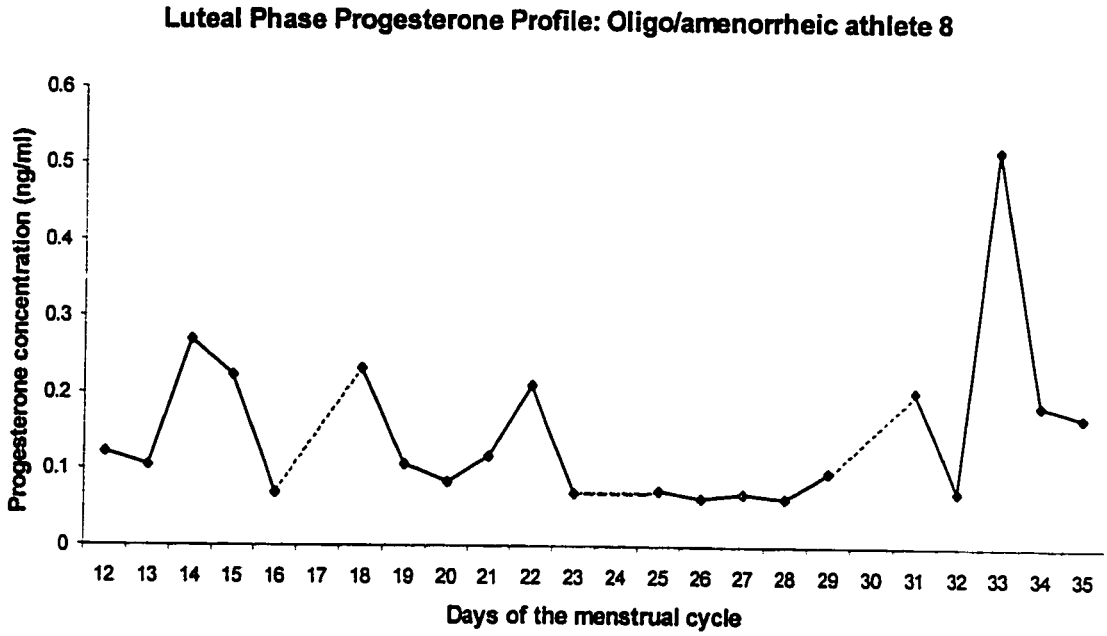
Individual Data for Oligo/amenorrheic Athlete 6

Athlete	Figure Skater
Chronological Age (years)	16
Gynecological Age (years)	3
Menstrual Cycle Status	Oligomenorrheic
Mean Progesterone Concentration	0.141 ng/ml
Peak Progesterone Concentration	0.327 ng/ml
Body Weight	48.4 kg
Height	157 cm
Sum of 5 Skinfolds	41.5 mm
Dietary Restraint Score	3.7
Mean Energy Intake	1294 kcal/day
Mean Energy Intake/kg	26.7 kcal/kg/day
% Calories from Carbohydrates	70%
% Calories from Protein	10%
% Calories from Fat	19%
REE	1263 kcal/day
Mean Activity Calories	435 kcal/day
Mean TEE	1787 kcal/day
Energy Balance	- 493 kcal/day



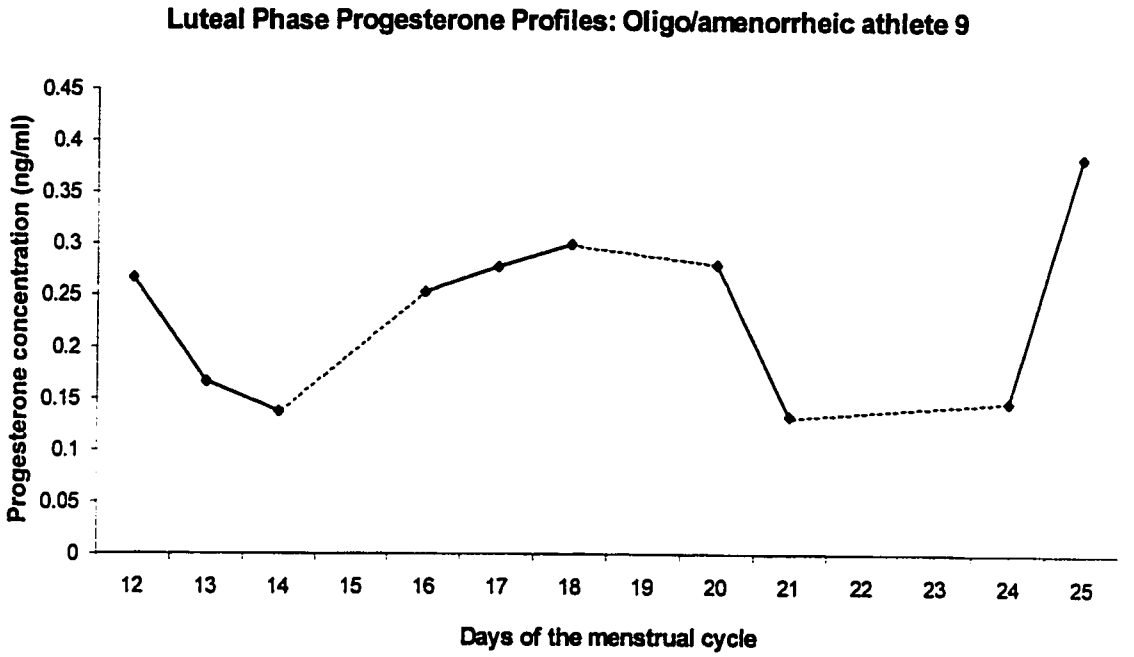
Individual Data for Oligo/amenorrheic Athlete 7

Athlete	Gymnast
Chronological Age (years)	15
Gynecological Age (years)	1
Menstrual Cycle Status	Oligomenorrheic
Mean Progesterone Concentration	0.157 ng/ml
Peak Progesterone Concentration	0.382 ng/ml
Body Weight	45.1 kg
Height	155 cm
Sum of 5 Skinfolds	52 mm
Dietary Restraint Score	2.6
Mean Energy Intake	2426 kcal/day
Mean Energy Intake/kg	53.8 kcal/kg/day
% Calories from Carbohydrates	58%
% Calories from Protein	15%
% Calories from Fat	26%
REE	1382 kcal/day
Mean Activity Calories	423 kcal/day
Mean TEE	1902 kcal/day
Energy Balance	534 kcal/day



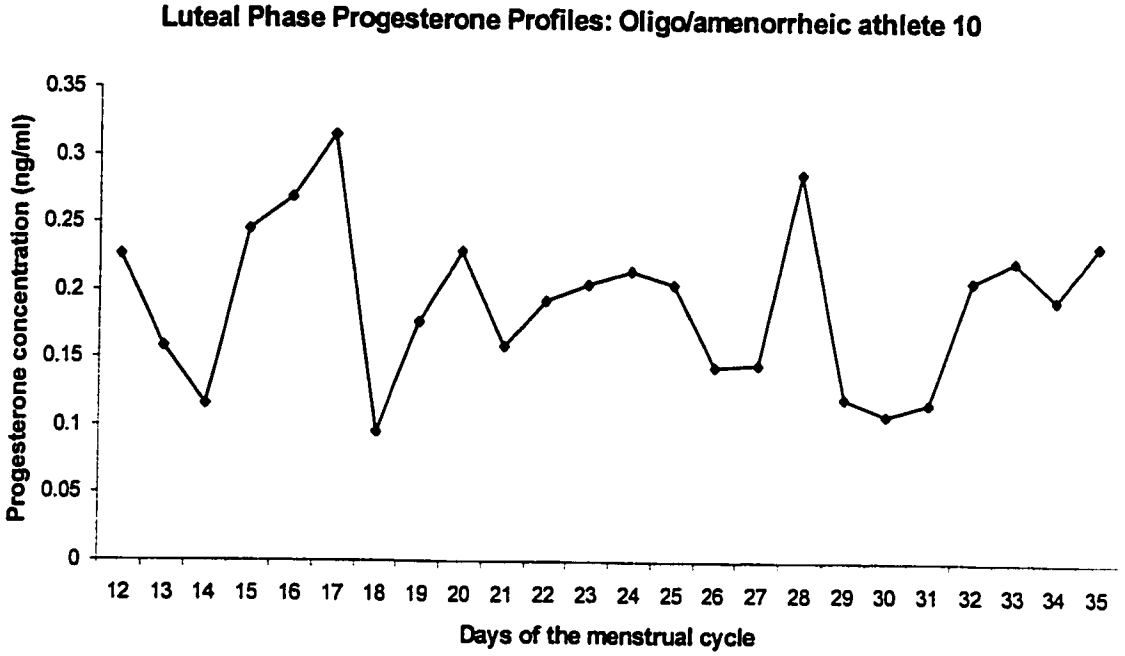
Individual Data for Oligo/amenorrheic Athlete 8

Athlete	Gymnast
Chronological Age (years)	15
Gynecological Age (years)	0.3
Menstrual Cycle Status	Amenorrheic
Mean Progesterone Concentration	0.148 ng/ml
Peak Progesterone Concentration	0.521 ng/ml
Body Weight	51.3 kg
Height	169 cm
Sum of 5 Skinfolds	54 mm
Dietary Restraint Score	1.0
Mean Energy Intake	2357 kcal/day
Mean Energy Intake/kg	45.9 kcal/kg/day
% Calories from Carbohydrates	64%
% Calories from Protein	12%
% Calories from Fat	24%
REE	1767 kcal/day
Mean Activity Calories	667 kcal/day
Mean TEE	2557 kcal/day
Energy Balance	- 200 kcal/day



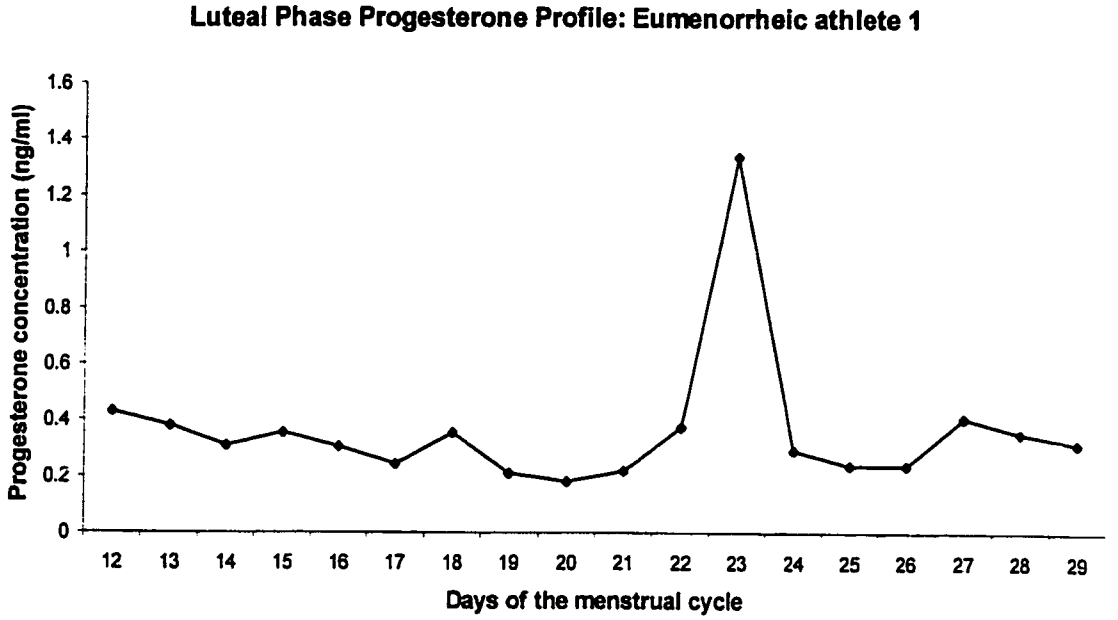
Individual Data for Oligo/amenorrheic Athlete 9

Athlete	Synchronized Swimmer
Chronological Age (years)	16
Gynecological Age (years)	2
Menstrual Cycle Status	Oligomenorrheic
Mean Progesterone Concentration	0.234 ng/ml
Peak Progesterone Concentration	0.385 ng/ml
Body Weight	56.4 kg
Height	162 cm
Sum of 5 Skinfolds	63.2 mm
Dietary Restraint Score	3.6
Mean Energy Intake	3148 kcal/day
Mean Energy Intake/kg	55.8 kcal/kg/day
% Calories from Carbohydrates	62%
% Calories from Protein	12%
% Calories from Fat	26%
REE	1900 kcal/day
Mean Activity Calories	1219 kcal/day
Mean TEE	3252 kcal/day
Energy Balance	- 104 kcal/day



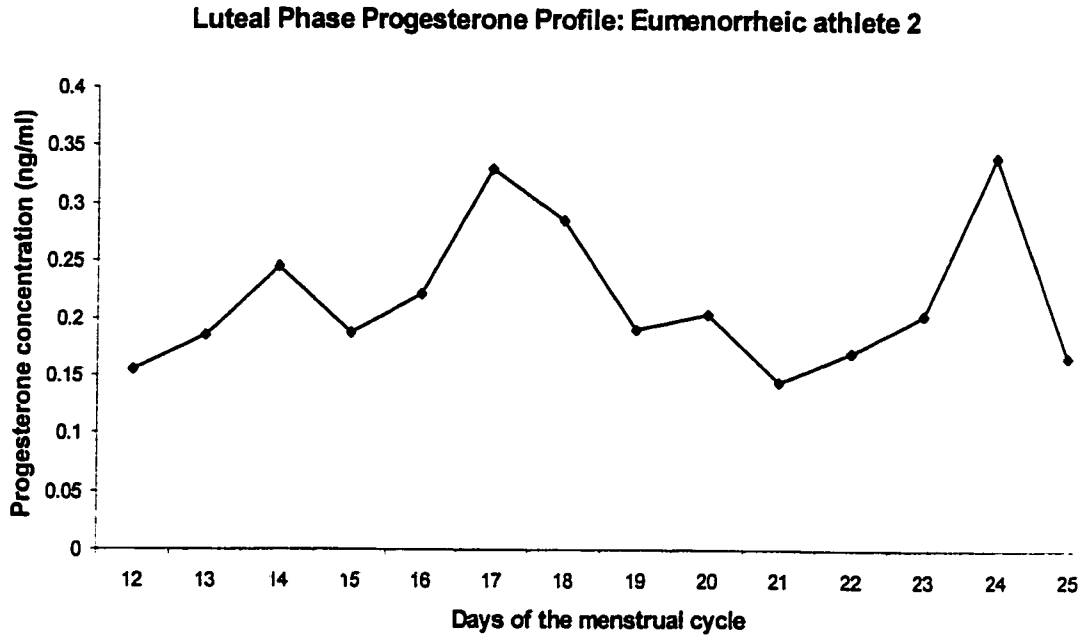
Individual Data for Oligo/amenorrheic Athlete 10

Athlete	Gymnast
Chronological Age (years)	16
Gynecological Age (years)	0.25
Menstrual Cycle Status	Amenorrheic
Mean Progesterone Concentration	0.191 ng/ml
Peak Progesterone Concentration	0.315 ng/ml
Body Weight	32.5 kg
Height	139 cm
Sum of 5 Skinfolds	30.7 mm
Dietary Restraint Score	1.1
Mean Energy Intake	1739 kcal/day
Mean Energy Intake/kg	53.5 kcal/kg/day
% Calories from Carbohydrates	54%
% Calories from Protein	14%
% Calories from Fat	32%
REE	1001 kcal/day
Mean Activity Calories	241 kcal/day
Mean TEE	1312 kcal/day
Energy Balance	427 kcal/day



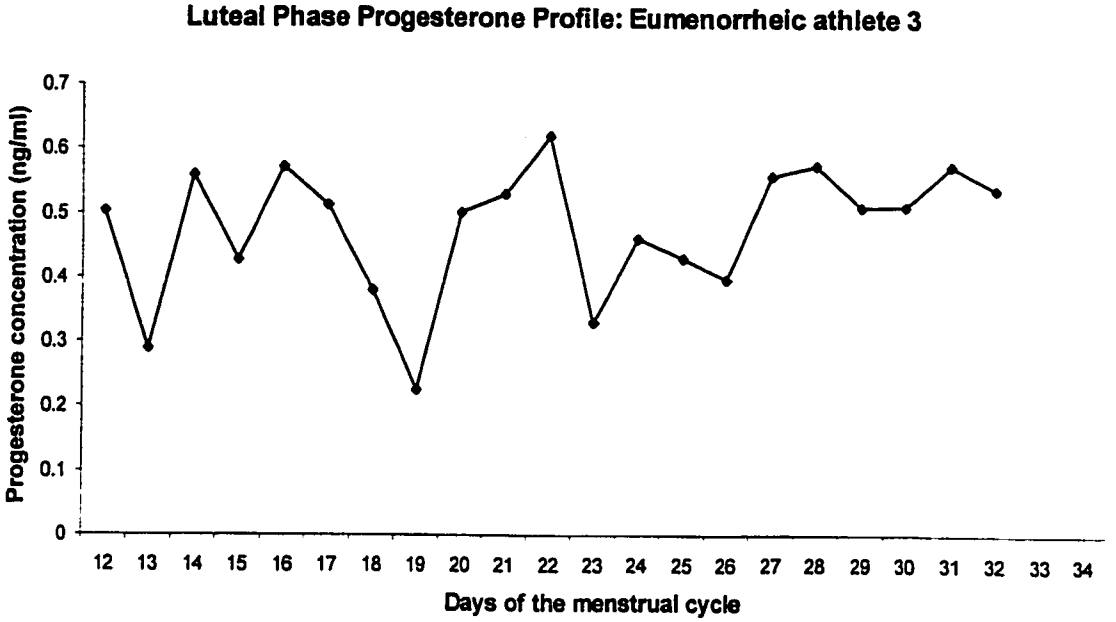
Individual Data for Eumenorrheic Athlete 1

Athlete	Gymnast
Chronological Age (years)	17
Gynecological Age (years)	2
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.365 ng/ml
Peak Progesterone Concentration	1.34 ng/ml
Body Weight	47.6 kg
Height	158 cm
Sum of 5 Skinfolds	43.2 mm
Dietary Restraint Score	2.9
Mean Energy Intake	1623 kcal/day
Mean Energy Intake/kg	34.1 kcal/kg/day
% Calories from Carbohydrates	62%
% Calories from Protein	14%
% Calories from Fat	24%
REE	1493 kcal/day
Mean Activity Calories	335 kcal/day
Mean TEE	1932 kcal/day
Energy Balance	- 309 kcal/day



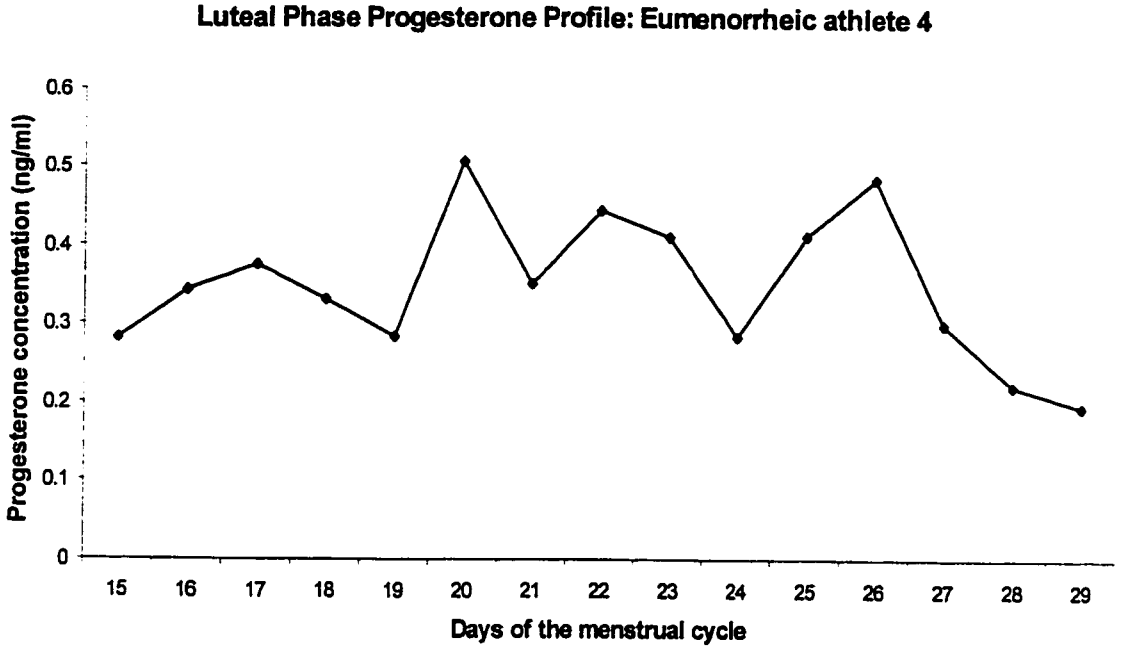
Individual Data for Eumenorrheic Athlete 2

Athlete	Gymnast
Chronological Age (years)	17
Gynecological Age (years)	5
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.216 ng/ml
Peak Progesterone Concentration	0.329 ng/ml
Body Weight	53.9 kg
Height	162 cm
Sum of 5 Skinfolds	61.2 mm
Dietary Restraint Score	2.2
Mean Energy Intake	2869 kcal/day
Mean Energy Intake/kg	53.2 kcal/kg/day
% Calories from Carbohydrates	59%
% Calories from Protein	11%
% Calories from Fat	30%
REE	1592 kcal/day
Mean Activity Calories	327 kcal/day
Mean TEE	2031 kcal/day
Energy Balance	838 kcal/day



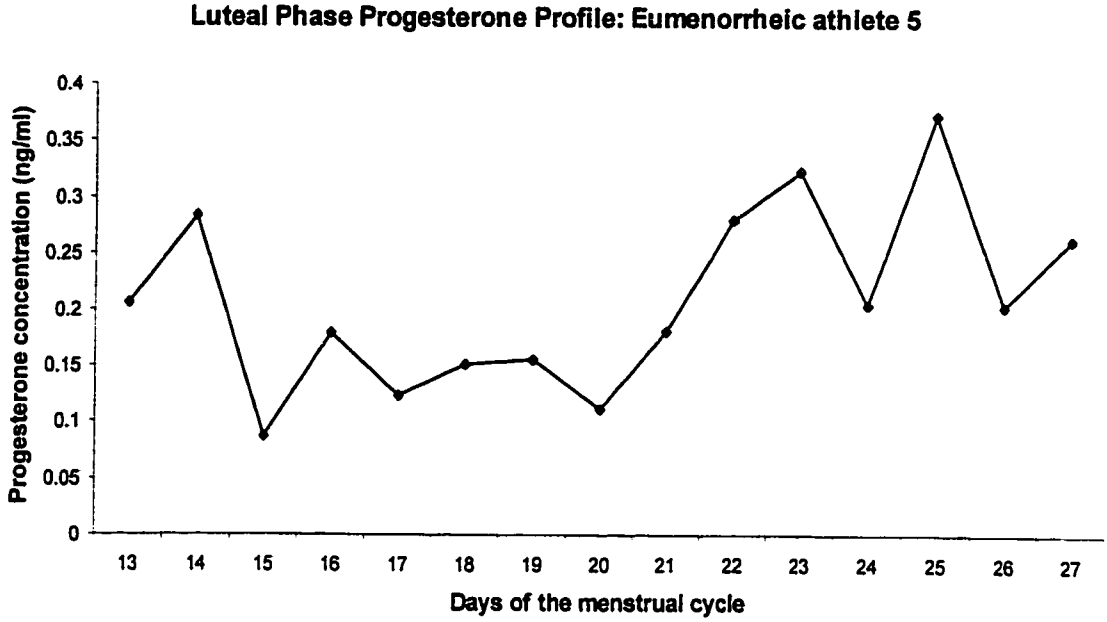
Individual Data for Eumenorrheic Athlete 3

Athlete	Figure Skater
Chronological Age (years)	15
Gynecological Age (years)	3
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.477 ng/ml
Peak Progesterone Concentration	0.620 ng/ml
Body Weight	54.2 kg
Height	163 cm
Sum of 5 Skinfolds	55.3 mm
Dietary Restraint Score	3.8
Mean Energy Intake	1723 kcal/day
Mean Energy Intake/kg	31.8 kcal/kg/day
% Calories from Carbohydrates	67%
% Calories from Protein	16%
% Calories from Fat	17%
REE	1631 kcal/day
Mean Activity Calories	518 kcal/day
Mean TEE	2263 kcal/day
Energy Balance	- 540 kcal/day



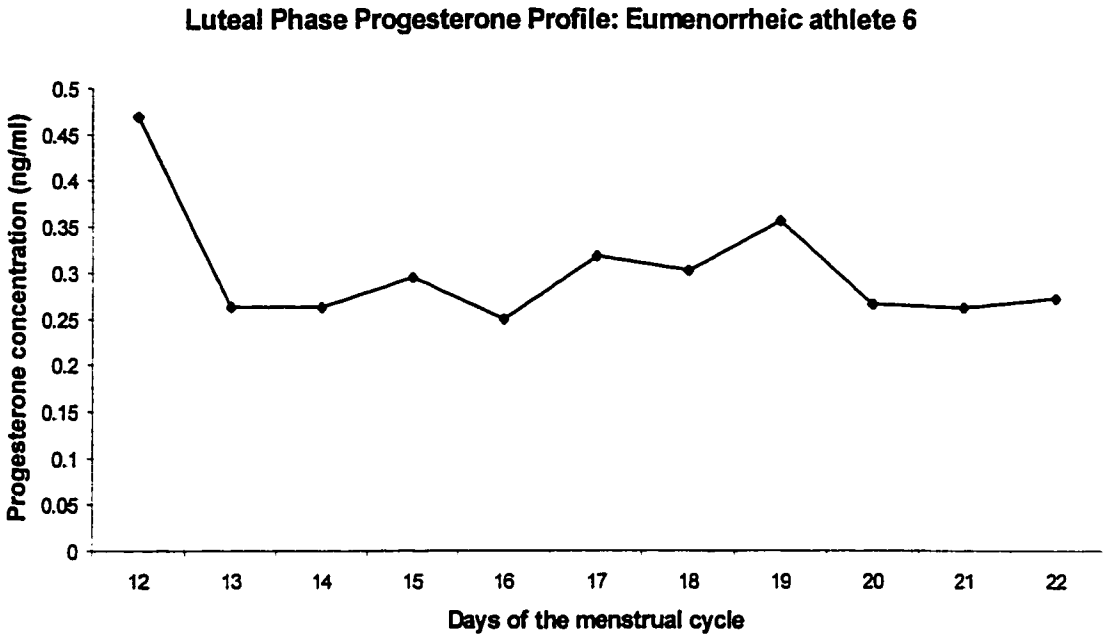
Individual Data for Eumenorrheic Athlete 4

Athlete	Figure Skater
Chronological Age (years)	17
Gynecological Age (years)	5
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.348 ng/ml
Peak Progesterone Concentration	0.507 ng/ml
Body Weight	55.7 kg
Height	160 cm
Sum of 5 Skinfolds	67.3 mm
Dietary Restraint Score	3.4
Mean Energy Intake	2011 kcal/day
Mean Energy Intake/kg	36.1 kcal/kg/day
% Calories from Carbohydrates	83%
% Calories from Protein	13%
% Calories from Fat	24%
REE	1397 kcal/day
Mean Activity Calories	413 kcal/day
Mean TEE	1908 kcal/day
Energy Balance	103 kcal/day



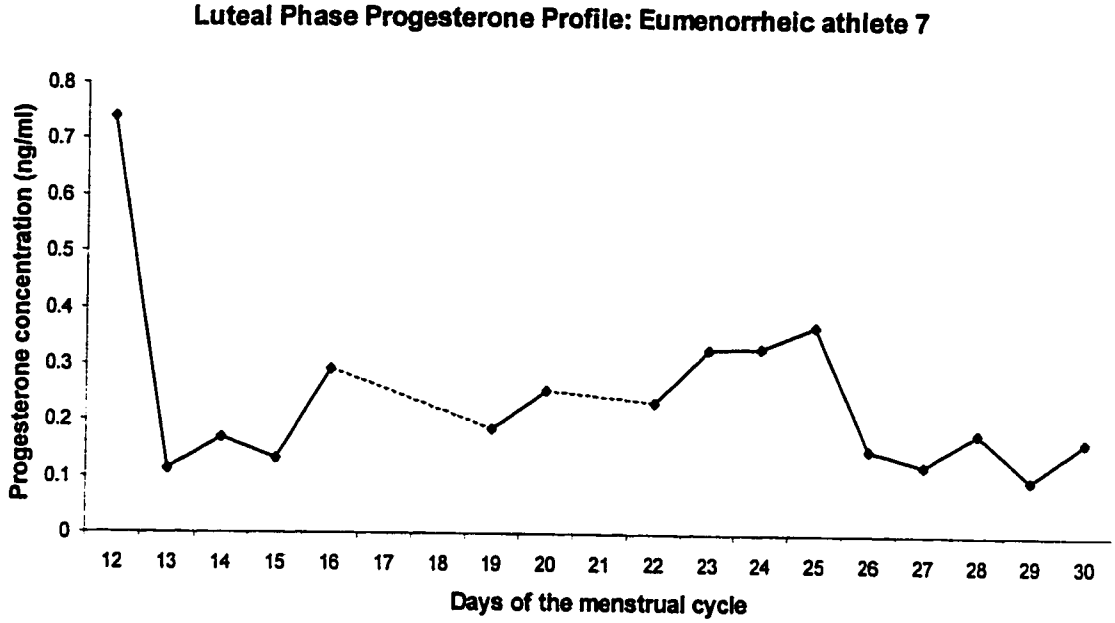
Individual Data for Eumenorrheic Athlete 5

Athlete	Gymnast
Chronological Age (years)	15
Gynecological Age (years)	1
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.208 ng/ml
Peak Progesterone Concentration	0.373 ng/ml
Body Weight	44.4 kg
Height	151 cm
Sum of 5 Skinfolds	55.8 mm
Dietary Restraint Score	1.2
Mean Energy Intake	2035 kcal/day
Mean Energy Intake/kg	45.8 kcal/kg/day
% Calories from Carbohydrates	54%
% Calories from Protein	13%
% Calories from Fat	33%
REE	1257 kcal/day
Mean Activity Calories	661 kcal/day
Mean TEE	2006 kcal/day
Energy Balance	29 kcal/day



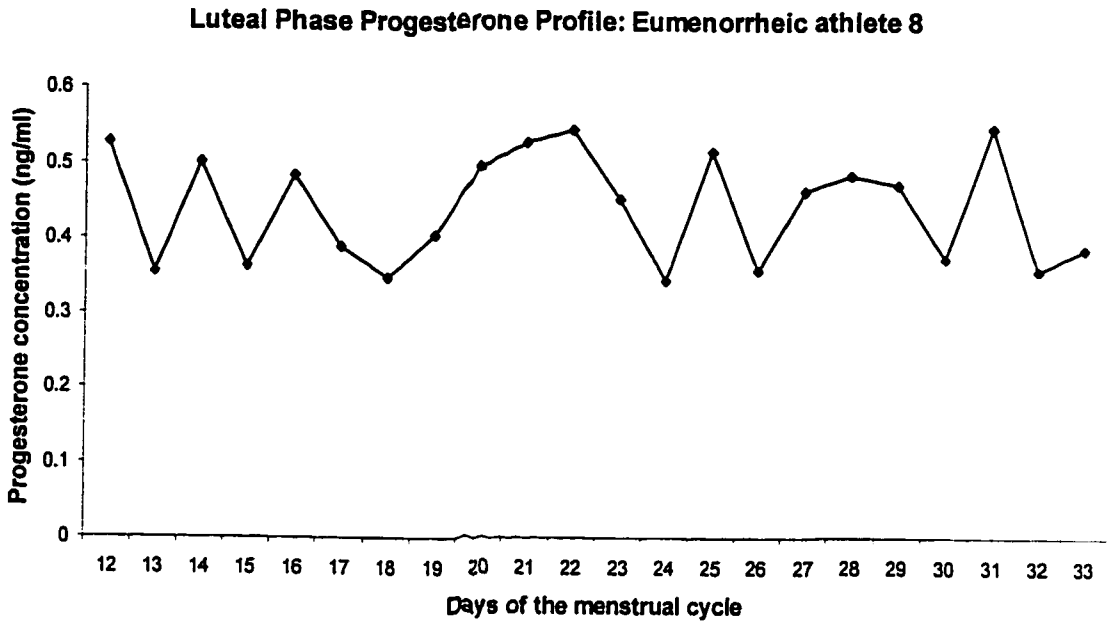
Individual Data for Eumenorrheic Athlete 6

Athlete	Figure Skater
Chronological Age (years)	16
Gynecological Age (years)	2
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.301 ng/ml
Peak Progesterone Concentration	0.469 ng/ml
Body Weight	58.3 kg
Height	168 cm
Sum of 5 Skinfolds	56.3 mm
Dietary Restraint Score	4.1
Mean Energy Intake	2314 kcal/day
Mean Energy Intake/kg	39.7 kcal/kg/day
% Calories from Carbohydrates	66%
% Calories from Protein	13%
% Calories from Fat	20%
REE	1475 kcal/day
Mean Activity Calories	736 kcal/day
Mean TEE	2314 kcal/day
Energy Balance	0



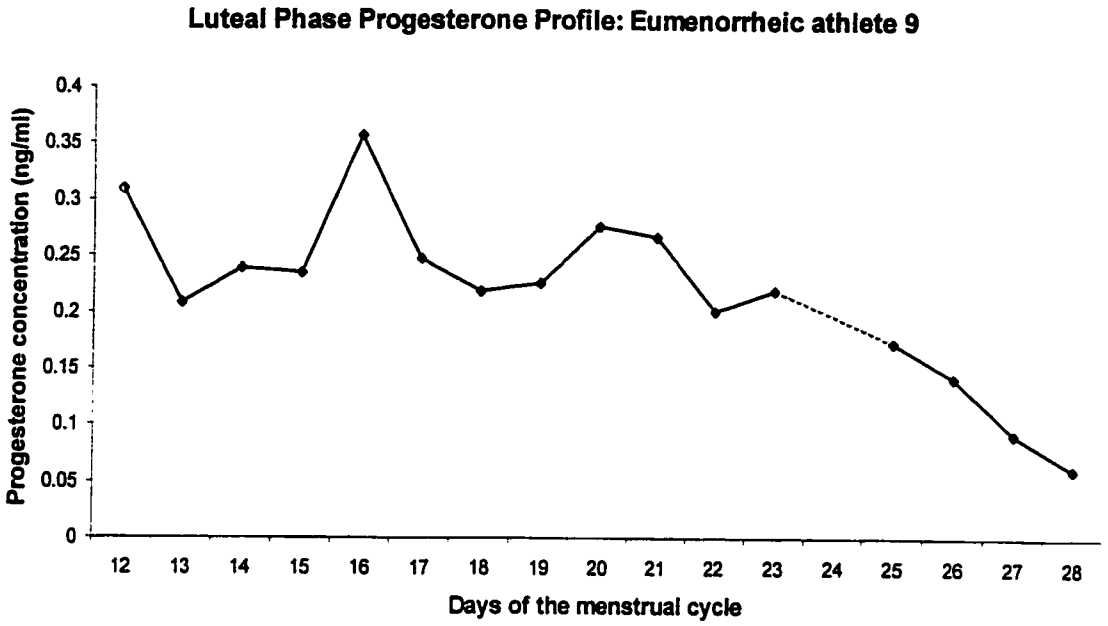
Individual Data for Eumenorrheic Athlete 7

Athlete	Gymnast
Chronological Age (years)	16
Gynecological Age (years)	2
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.242 ng/ml
Peak Progesterone Concentration	0.740 ng/ml
Body Weight	47.9 kg
Height	159 cm
Sum of 5 Skinfolds	73.6 mm
Dietary Restraint Score	1.5
Mean Energy Intake	1638 kcal/day
Mean Energy Intake/kg	34.2 kcal/kg/day
% Calories from Carbohydrates	58%
% Calories from Protein	13%
% Calories from Fat	29%
REE	1340 kcal/day
Mean Activity Calories	506 kcal/day
Mean TEE	2017 kcal/day
Energy Balance	- 380 kcal/day



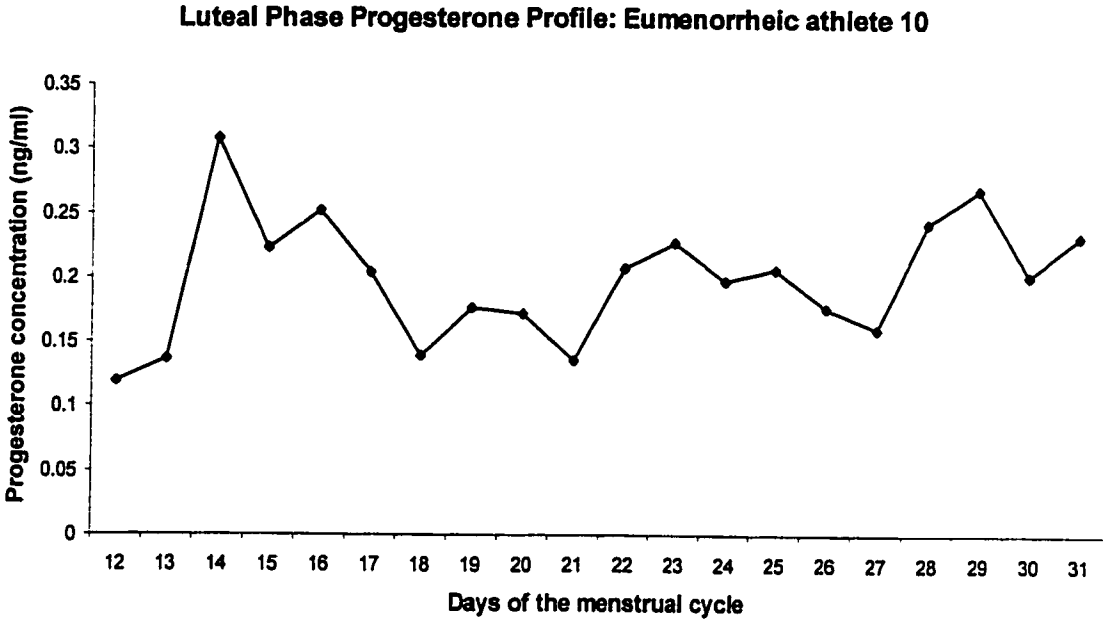
Individual Data for Eumenorrheic Athlete 8

Athlete	Figure Skater
Chronological Age (years)	16
Gynecological Age (years)	3
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.441 ng/ml
Peak Progesterone Concentration	0.528 ng/ml
Body Weight	58.5 kg
Height	161 cm
Sum of 5 Skinfolds	64.7 mm
Dietary Restraint Score	3.6
Mean Energy Intake	1762 kcal/day
Mean Energy Intake/kg	30.1 kcal/kg/day
% Calories from Carbohydrates	73%
% Calories from Protein	13%
% Calories from Fat	14%
REE	1486 kcal/day
Mean Activity Calories	597 kcal/day
Mean TEE	2187 kcal/day
Energy Balance	- 425 kcal/day



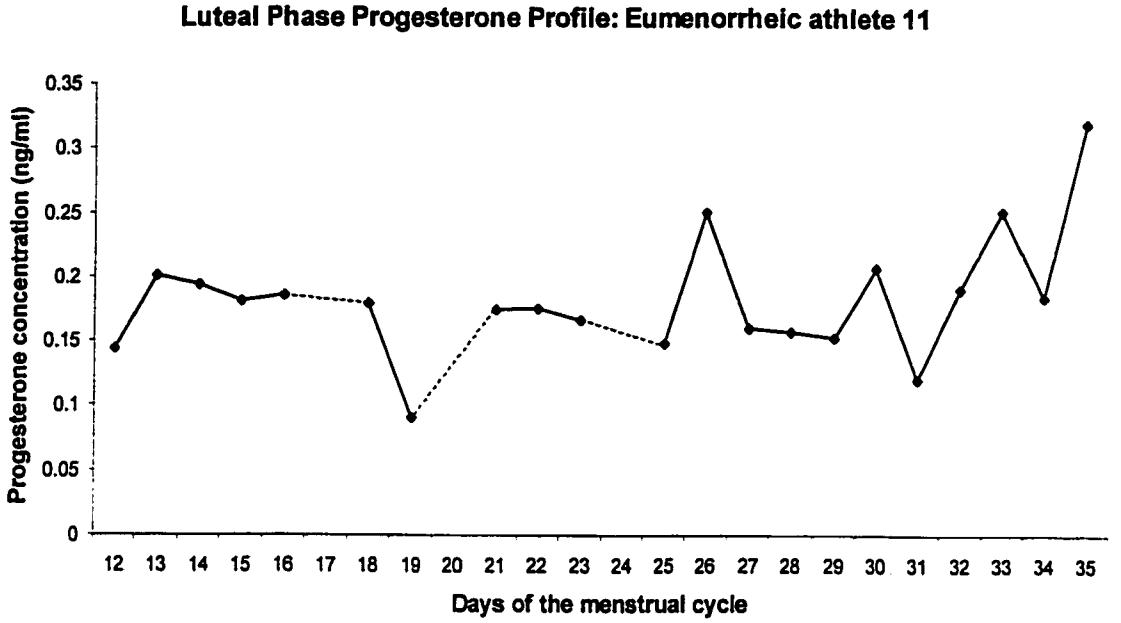
Individual Data for Eumenorrheic Athlete 9

Athlete	Figure Skater
Chronological Age (years)	18
Gynecological Age (years)	3
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.217 ng/ml
Peak Progesterone Concentration	0.357 ng/ml
Body Weight	56.4 kg
Height	162 cm
Sum of 5 Skinfolds	69.4 mm
Dietary Restraint Score	2.1
Mean Energy Intake	2035 kcal/day
Mean Energy Intake/kg	36.1 kcal/kg/day
% Calories from Carbohydrates	56%
% Calories from Protein	16%
% Calories from Fat	28%
REE	1472 kcal/day
Mean Activity Calories	425 kcal/day
Mean TEE	2000 kcal/day
Energy Balance	35 kcal/day



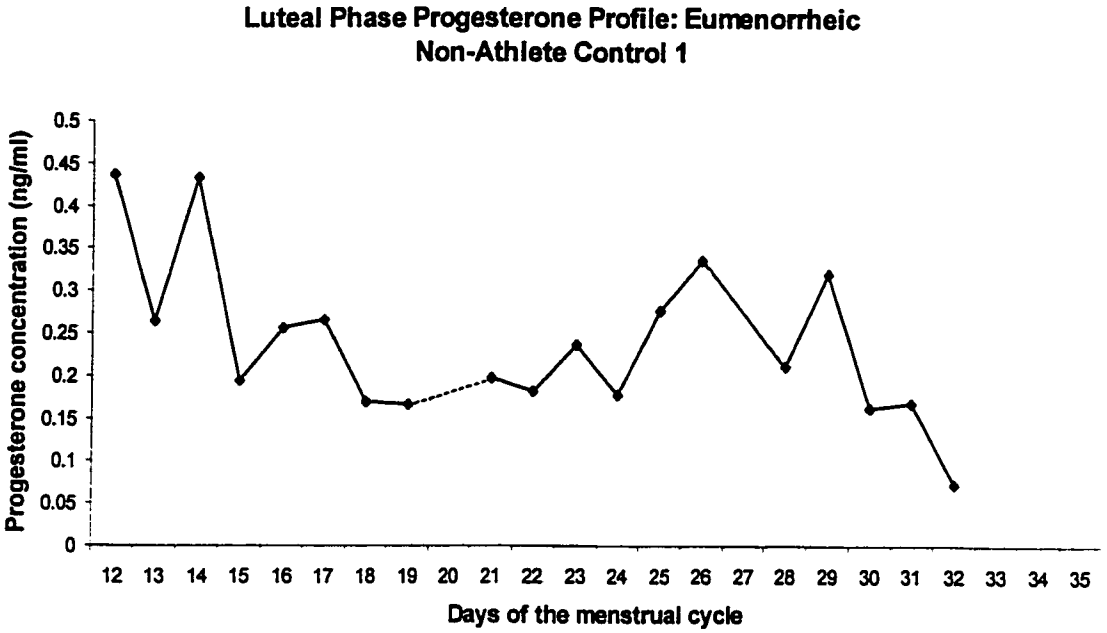
Individual Data for Eumenorrheic Athlete 10

Athlete	Synchronized Swimmer
Chronological Age (years)	18
Gynecological Age (years)	5
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.199 ng/ml
Peak Progesterone Concentration	0.307 ng/ml
Body Weight	49.4 kg
Height	159 cm
Sum of 5 Skinfolds	47.8 mm
Dietary Restraint Score	1.5
Mean Energy Intake	2240 kcal/day
Mean Energy Intake/kg	45.3 kcal/kg/day
% Calories from Carbohydrates	60%
% Calories from Protein	13%
% Calories from Fat	27%
REE	1447 kcal/day
Mean Activity Calories	896 kcal/day
Mean TEE	2444 kcal/day
Energy Balance	- 204 kcal/day



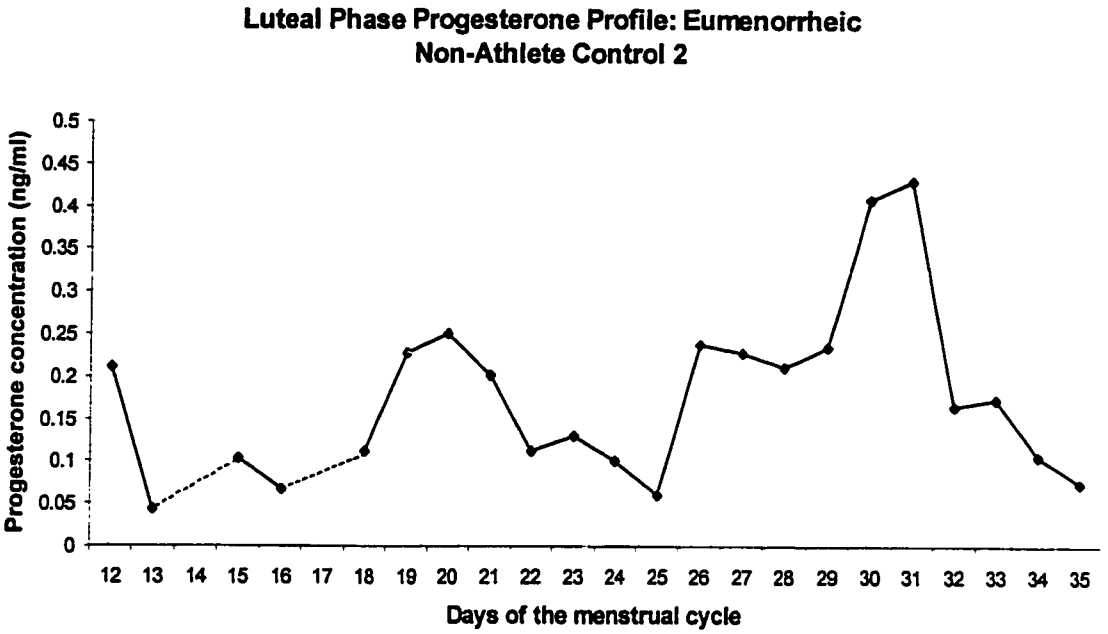
Individual Data for Eumenorrheic Athlete 11

Athlete	Gymnast
Chronological Age (years)	16
Gynecological Age (years)	2
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.182 ng/ml
Peak Progesterone Concentration	0.321 ng/ml
Body Weight	57.4 kg
Height	162 cm
Sum of 5 Skinfolds	67 mm
Dietary Restraint Score	3.4
Mean Energy Intake	2592 kcal/day
Mean Energy Intake/kg	45.1 kcal/day
% Calories from Carbohydrates	57%
% Calories from Protein	11%
% Calories from Fat	29%
REE	1049 kcal/day
Mean Activity Calories	666 kcal/day
Mean TEE	1789 kcal/day
Energy Balance	803 kcal/day



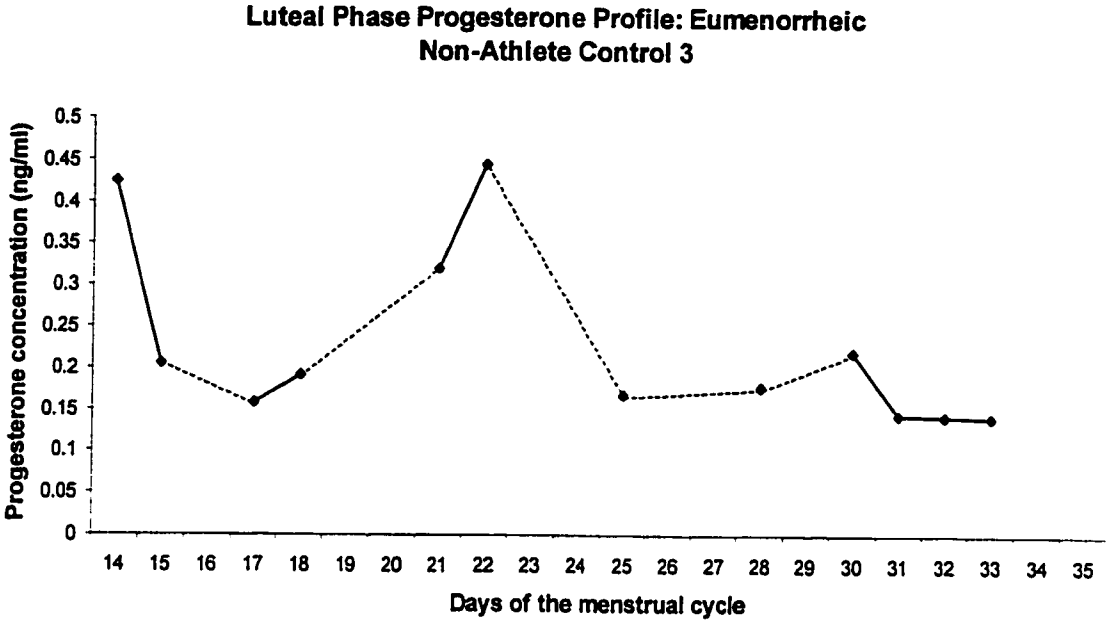
Individual Data for Eumenorrheic Non-Athlete Control 1

Chronological Age (years)	15
Gynecological Age (years)	2
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.238 ng/ml
Peak Progesterone Concentration	0.437 ng/ml
Body Weight	62.6 kg
Height	175 cm
Sum of 5 Skinfolds	83.3 mm
Dietary Restraint Score	1.3
Mean Energy Intake	2593 kcal/day
Mean Energy Intake/kg	41.1 kcal/kg/day
% Calories from Carbohydrates	44%
% Calories from Protein	14%
% Calories from Fat	42%
REE	1519 kcal/day
Mean Activity Calories	237 kcal/day
Mean TEE	1862 kcal/day
Energy Balance	731 kcal/day



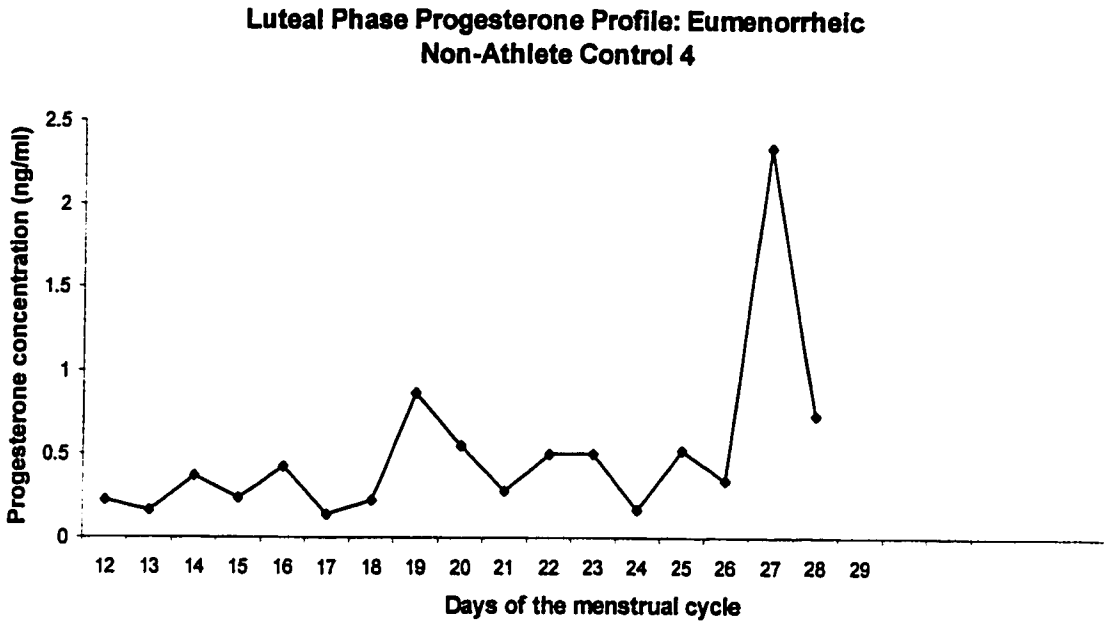
Individual Data for Eumenorrheic Non-Athlete Control 2

Chronological Age (years)	17
Gynecological Age (years)	6
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.176 ng/ml
Peak Progesterone Concentration	0.431 ng/ml
Body Weight	68.4 kg
Height	179 cm
Sum of 5 Skinfolds	97.5 mm
Dietary Restraint Score	2.0
Mean Energy Intake	1503 kcal/day
Mean Energy Intake/kg	22.0 kcal/kg/day
% Calories from Carbohydrates	45%
% Calories from Protein	15%
% Calories from Fat	40%
REE	1493 kcal/day
Mean Activity Calories	147 kcal/day
Mean TEE	1745 kcal/day
Energy Balance	- 242 kcal/day



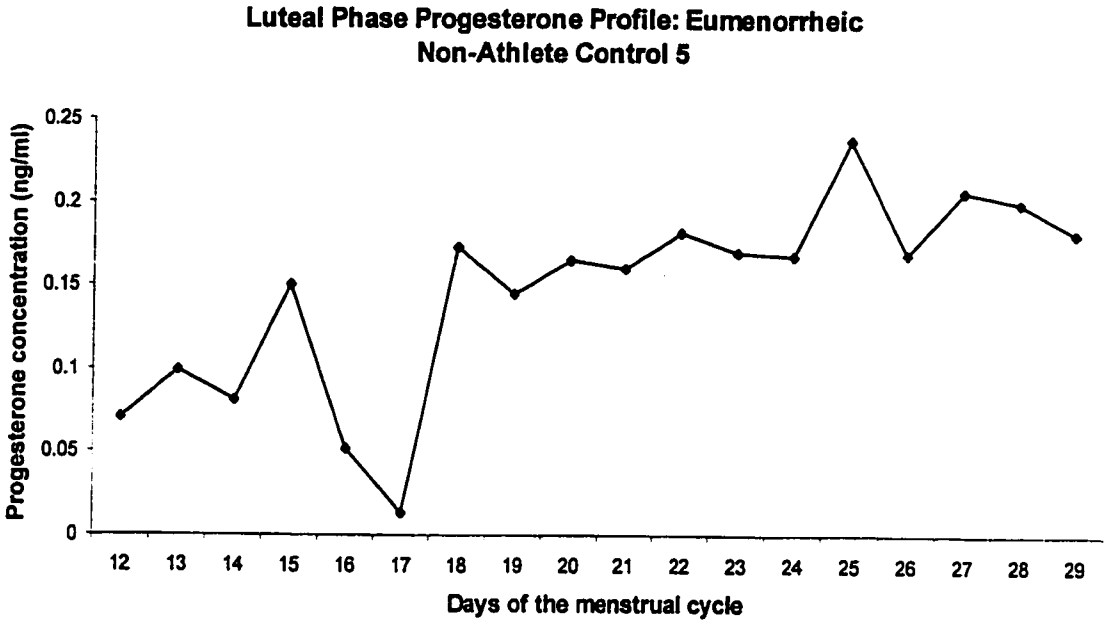
Individual Data for Eumenorrheic Non-Athlete Control 3

Chronological Age (years)	17
Gynecological Age (years)	4
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.228 ng/ml
Peak Progesterone Concentration	0.425 ng/ml
Body Weight	65 kg
Height	168 cm
Sum of 5 Skinfolds	61 mm
Dietary Restraint Score	2.1
Mean Energy Intake	1851 kcal/day
Mean Energy Intake/kg	28.5 kcal/kg/day
% Calories from Carbohydrates	58%
% Calories from Protein	13%
% Calories from Fat	29%
REE	1631 kcal/day
Mean Activity Calories	322 kcal/day
Mean TEE	2067 kcal/day
Energy Balance	- 216 kcal/day



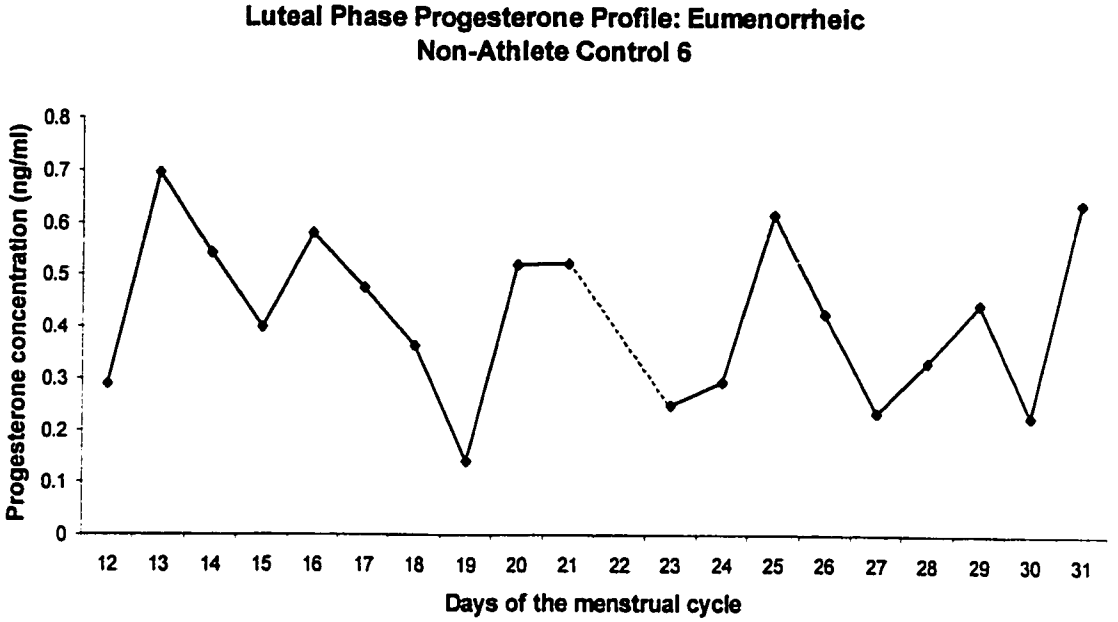
Individual Data for Eumenorrheic Non-Athlete Control 4

Chronological Age (years)	16
Gynecological Age (years)	4
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.505 ng/ml
Peak Progesterone Concentration	2.34 ng/ml
Body Weight	64.2 kg
Height	169 cm
Sum of 5 Skinfolds	112 mm
Dietary Restraint Score	2.3
Mean Energy Intake	1948 kcal/day
Mean Energy Intake/kg	30.3 kcal/kg/day
% Calories from Carbohydrates	59%
% Calories from Protein	12%
% Calories from Fat	29%
REE	1538 kcal/day
Mean Activity Calories	234 kcal/day
Mean TEE	1880 kcal/day
Energy Balance	67 kcal/day



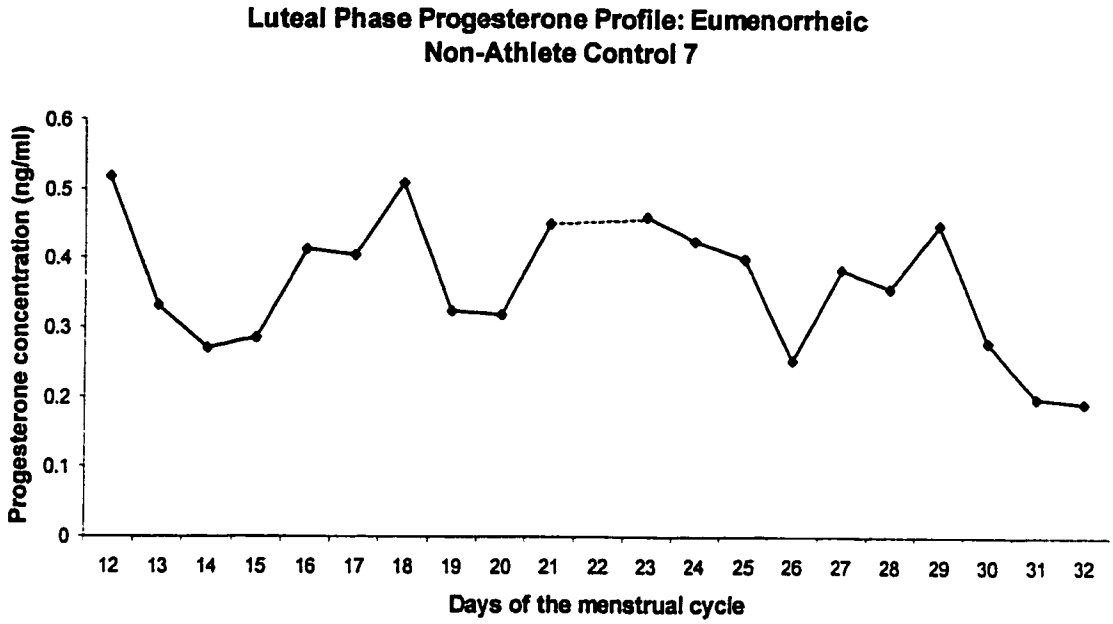
Individual Data for Eumenorrheic Non-Athlete Control 5

Chronological Age (years)	15
Gynecological Age (years)	2
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.146 ng/ml
Peak Progesterone Concentration	0.207 ng/ml
Body Weight	80.5 kg
Height	174 cm
Sum of 5 Skinfolds	159 mm
Dietary Restraint Score	2.5
Mean Energy Intake	2937 kcal/day
Mean Energy Intake/kg	36.5 kcal/kg/day
% Calories from Carbohydrates	44%
% Calories from Protein	15%
% Calories from Fat	41%
REE	1660 kcal/day
Mean Activity Calories	233 kcal/day
Mean TEE	2009 kcal/day
Energy Balance	928 kcal/day



Individual Data for Eumenorrheic Non-Athlete Control 6

Chronological Age (years)	16
Gynecological Age (years)	5
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.419 ng/ml
Peak Progesterone Concentration	0.695 ng/ml
Body Weight	60.1 kg
Height	160 cm
Sum of 5 Skinfolds	117 mm
Dietary Restraint Score	2.8
Mean Energy Intake	2908 kcal/day
Mean Energy Intake/kg	48.4 kcal/kg/day
% Calories from Carbohydrates	51%
% Calories from Protein	14%
% Calories from Fat	35%
REE	1485 kcal/day
Mean Activity Calories	375 kcal/day
Mean TEE	1964 kcal/day
Energy Balance	944 kcal/day



Individual Data for Eumenorrheic Non-Athlete Control 7

Chronological Age (years)	18
Gynecological Age (years)	5
Menstrual Cycle Status	Eumenorrheic
Mean Progesterone Concentration	0.361 ng/ml
Peak Progesterone Concentration	0.519 ng/ml
Body Weight	64.6 kg
Height	176 cm
Sum of 5 Skinfolds	82.1 mm
Dietary Restraint Score	2.3
Mean Energy Intake	1418 kcal/day
Mean Energy Intake/kg	21.9 kcal/kg/day
% Calories from Carbohydrates	55%
% Calories from Protein	15%
% Calories from Fat	30%
REE	1506 kcal/day
Mean Activity Calories	262 kcal/day
Mean TEE	1873 kcal/day
Energy Balance	- 456 kcal/day