THE MENSTRUAL CYCLE, OVULATORY, AND HORMONAL EFFECTS OF AN 8-WEEK ABRUPTLY INCREASING RUNNING PROGRAM IN RECREATIONALLY ACTIVE WOMEN

by

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ABSTRACT

Background: Researchers assert that a woman's reproductive system is disrupted below an energy availability (EA) threshold of 25kcal/kgLBM/day, independent of exercise stress. Alternatively, research also suggests that an abrupt increase in training over an 8-week period disrupts luteal and ovulatory function in recreationally active women. It is not known whether EA and/or the stress of an abrupt program will further disrupt the luteal, ovulatory, and hormone function in gynecological mature women with regularly ovulatory and disturbed menstrual cycles.

Methods: The menstrual cycle, ovulatory, and hormonal characteristics in recreationally active and gynecologically mature women (19 +/- 0.8 years) were prospectively compared between a 2-menstrual cycle control phase and an 8-week abruptly increasing training phase. Women with normally ovulatory and menstrual cycle and ovulatory disturbances were included in this study. The twenty participants sustained no injuries, as the 8-week training program increased abruptly in volume and intensity by a mean rate of 19.5% per week. Initial aerobic capacity (37.6 +/- 3ml/kg/min) non-significantly increased by 2.3ml/kg/min ($p = 0.086$) and ventilatory threshold significantly increased by 2ml/kg/min ($p = 0.021$). Regardless of a significant decrease in EA by 4.8kcal/kgLBM/day, body weight and composition remained constant over the training phase.

Results: The Menstrual Cycle Diaries© and Quantitative Basal Temperatures (QBT) method of least-squares analysis revealed no significant training differences in menstrual cycle, luteal phase, and follicular phase length, as well as, ovulation status, luteal phase index, and pre-menstrual symptoms. Competitive enzyme immunoassays (EIAs) technique revealed no statistical control-to-training-phase differences in early follicular phase pregnanediol-3-glucuronide (PdG), luteal phase PdG and estrone conjugates (E1C), and cortisol. The control to training change in early follicular phase E1C did approach statistical significance ($p = 0.0012$) and the change in energy availability accounted for 23.1% of its variability ($p = 0.044$).

Conclusions: Menstrual cycle and ovulatory characteristics in recreationally active women are robust to an 8-week abruptly increasing training program and a mean decrease in energy availability by 4.8kcal/kgLBM/day. However, a change in energy availability by 4.8kcal/kgLBM/day accounted for 23.1% of the variance in early follicular phase E1C change from the control menstrual cycle #2 to training menstrual cycle #2.

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Definitions

The following menstrual cycle parameters were defined based on the literature (Vollman, 1977 and Prior, 1996a).

Menstrual cycle length: The length was defined as the number of days from the onset of menstruation to the day prior to the next menstruation. The onset of menstruation in menstrual cycles experiencing spotting was defined as the first day of spotting, if the spotting occurred on consecutive days leading up to flow. A normal menstrual cycle length is between 21 and 35 days.

Ovulatory: Ovulatory cycle is one in which ovulation (release of an egg) occurs. In this study, ovulatory cycles are distinguished by an increase in basal body temperature of approximately 0.33°C due to the rise in serum progesterone levels equal to or above 5ng/ml or 18 mnol/L (Prior, Vigna, Schulzer, Hall, & Bonen, 1990). An anovulatory cycle is one in which no egg is released. In this study, anovulatory cycles display "a monophasic set of basal temperatures during one cycle in which the least squares program, Maximina©, detects no significant shift" (Petit and Prior, 2000; pg.139).

Follicular Phase: The onset of the follicular phase is the first day of flow and the end is the day of the luteal onset, as determined by Maximina©.

Luteal Phase Length: The luteal phase length is defined by the mean shift in basal body temperature ($p < 0.05$) until the day before the onset of menstrual flow (10 to 16 days). A shortened luteal phase has a length less than 10 days.

Luteal Phase Index: The proportion of the luteal phase length to the length of the menstrual cycle is defined as the luteal phase index (LPI). This variable is important, as Prior, Vigna, Schechter, & Burgess (1990) found this index to be strongly correlated to the changes in bone density experienced by the 66 women ($r = 0.54$, $p < 0.001$). The index is reported as a decimal, for example a 14-day luteal phase in a 28-day cycle would yield a luteal phase index of 0.5.

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Chapter I Overview

1.1 Introduction

Subtle menstrual cycle adaptations are more prevalent among physically active women compared with their less active counterparts. These adaptive changes in menstrual cycle and reproductive hormones include decreased pre-menstrual symptoms, inadequate production of luteal phase progesterone, shortened luteal phases, anovulation, and lengthening of the menstrual cycle.

Ascertaining causal relationships, if present, among exercise training, weight, weight change, reproductive maturation, situational stresses, and eating attitudes with reproductive disturbances is very important. This is because these disturbances increase an active woman's health risks, primarily for low bone density, infertility, and spontaneous abortions (Prior, 1997 and DeSouza, 2003). Potential etiological factors among others, include the stress of physical activity, acute weight loss, weight cycling, lesser or greater ideal body weight, energy deficit, emotional distress, physical illness, sleep disruption, cognitive dietary restraint, eating disorders, and infections (Prior 1996a; Loucks & Horvath 1985; Mclean, Barr, & Prior, 2001; Loucks, Verdun, & Heath, 1998). In the existing literature relating to physically active women, two potentially conflicting theories exist: restricted energy availability and the stress of exercise. The stress of exercise refers to "the process of stressing an organism at a higher level than before in order to provide a stimulus for adaptation and supercompensation..." (Fry, Morton, & Keast, 1992; pg 242).

Current literature asserts that balanced energy availability, not the stress of exercise, is the vital link in maintaining a healthy reproductive system in active women (Loucks et al. 1998; Loucks & Verdun, 1998; and Loucks & Thuma, 2003; DeSouza 2003; Williams, Caston-Balderrama, Helmreich, Parfitt, Nosbisch, & Cameron, 2001). Substantial evidence supports a relationship between energy availabilities below DeSouza's et al. (1998) finding of 24.7 kilocalories per kilogram of lean body mass per day (kcal/kgLBM/day) and menstrual cycle disturbances; however, a detailed review of the energy availability findings above 25kcal/kgLBM/day reveal subtle and noteworthy disagreements (DeSouza, Miller, Loucks, Luciano, Pescatello, Campbell, & Lasley, 1998; Loucks et al., 1998, Loucks & Verdun, 1998; and Loucks & Thuma, 2003). Furthermore, the research insufficiently and inaccurately reproduces the exercise stress of training. The role of a stressful training regimen in energy-balanced women with menstrual cycle and ovulatory disturbances remains inconclusive.

Specifically, Loucks et al. (1998); Loucks & Verdun (1998); and Loucks & Thuma's (2003) research imposed a stressful exercise regime over a 4-day period; thus, limiting the conclusions to short-term exercise effects. The amount of stress imposed by Williams' and DeSouza's training programs remain speculative, as Williams, Caston-Balderrama, et al. (2001) in the non-human primate model gradually increased the cynomolgus monkey's training at their own comfort level and DeSouza's et al. (1998) cross-sectional study in recreationally active women maintained a constant volume of exercise over 3 months. Moreover, the energy deficit findings are limited in evaluating the role of exercise, as each study failed to report any measurements of increasing exercise load and hence its ability to produce a physiological stress.

In addition to the energy availability literature, the exercise stress research also remains inconclusive in establishing a relationship between training and subtle menstrual cycle and ovulatory disturbances. Similarly to Williams and DeSouza, the current available research fails to sustain a training stimulus by either maintaining constant training volumes (Bonen, 1992) or by increasing gradually (Rogol et al., 1992 and Prior, Vigna, Schechter, et al., 1990). Bullen, Skrinar, Beitins, Gretchen von Mering, Turnbull, & McArthur (1985) and Williams, Bullen, McArthur, Skrinar, & Turnball (1999) successfully observed menstrual cycle disturbances by imposing an abruptly increasing training stimulus; however, both studies were confounded by the energy deficit variable. In order to assess the component of physical activity, independently of energy deficit, a measurable physical activity load must be imposed over a sufficiently long period of time. Specifically, concurrently manipulating increasing training rates, abruptly and/or a gradually, while monitoring energy availability will add integral knowledge to existing literature.

The above energy deficit and exercise stress studies documenting significant disturbances in menstrual cycle and ovulatory function have explored untrained women with a mean gynecological age of 9.4 years, ranging from 7.7 to 14.9 years (Bullen et al, 1985; Williams et al., 1999; Loucks et al. 1998; Loucks & Verdun, 1998; Loucks & Heath, 1994; Loucks & Thuma, 2003; DeSouza et al., 1998). Gynecological age is defined as the number of years past menarche. This mean gynecological age of 9.4 years is more susceptible to menstrual cycle and ovulatory disturbances. Vollman (1977) documented that regular ovulatory cycles with normal luteal phase lengths are most robust between the gynecological ages of 12 and 30 years. In addition, the studies have only investigated women with normal menstrual and ovulatory cycle (Bullen et al, 1985; Williams et al., 1999; Loucks et al. 1998; Loucks & Verdun, 1998; Loucks & Heath, 1994; and Loucks & Thuma, 2003).

This study will be the first to explore exercise training effects in gynecologically mature and recreationally active women with normal menstrual cycles and menstrual cycle and ovulatory disturbances. The present study will also be the first to address a segment of the unexplored notion of the rate of exercise program increases while monitoring energy availability. The main objective is to determine whether an 8-week abruptly increasing training program will decrease pre-menstrual symptoms and luteal phase progesterone production, shorten luteal phases, result in anovulatory cycles, and/or lengthen the menstrual cycle. Exercise stress in this study refers to the physical stimulus of abruptly increasing the volume and intensity by 22.5% per week over an 8-week period and it will be quantified using the training monitoring form (McKenzie, 1995) (Appendix B). This study will add to the existing energy deficit and exercise stress research by monitoring energy availability while imposing a stressful training program over a sufficiently long period of time in gynecologically mature women.

1.2 Statement of the Problem

1) A relationship, independent of exercise stress, appears to exist between luteinizing hormone (LH) pulsatility and luteal function in relation to energy availabilities below 25kcal/kgLBM/day. However, inter- and intra-study comparisons reveal conflicting results in reference to energy availabilities above 25 kcal/kgLBM/day and menstrual cycle and/or ovulatory disturbances. Research has yet to resolve whether the existing incongruencies in energy-balanced women are methodological or the result of an alternative mechanism.

2) To date, there has been no prospective human study exploring Hans Seyle's (1939) observations that rats develop anestrus while following an abruptly increasing training regimen. Furthermore, a causal relationship between training and amenorrhea has not been established in prospective investigations with physically active woman.

3) An inter-study comparison of the rate of increases in long-term (8-weeks-to-a-year) training programs reveals that a potential relationship between abruptly increasing training programs and the more subtle ovulatory disturbances of decreased pre-menstrual symptoms, shortened luteal phases, and anovulatory cycles. This notion of abruptly increasing training program over a sufficient period of time has yet to be scientifically explored and quantified in gynecologically mature women, independent of energy availability.

1.3 Purpose of the Investigation

The primary purpose of this study was to determine the effects of an 8-week abruptly increasing running program on the menstrual cycle parameters of luteal phase length, ovulation status, menstrual cycle length, luteal phase index (luteal phase length divided by menstrual cycle length), and pre-menstrual symptoms in women with normally ovulatory cycles, as well as, in women with initial menstrual cycle and ovulatory disturbances. Additionally, the hormonal changes of pregnanediol-3-glucuronide (PdG), estrone conjugates (E1C), and cortisol were compared from the second "control" menstrual cycle to the "training program" menstrual cycles.

The secondary purpose was to contribute valuable knowledge to a future in-depth analysis concerning the effects of different rates of training program increases on the menstrual cycle and ovulatory outcomes in gynecologically mature women. In particular, the research project served as a pilot project to address the following issues:

A) The number of drop-outs, as well as the number and types of injuries that occur during the 8-week abruptly increasing running program;
B) The ability to recruit gynecologically mature women with two consecutive 21 to 35 days in length, ovulatory cycles with a normal luteal phase length between 10 to 16 days.
C) Further clarify any existing relationships between the training and/or nutritional intake variables in conjunction with the menstrual cycle and ovulatory disturbances.

1.4 Hypotheses

In energy deficit participants:

1) The menstrual cycle and ovulatory, as well as hormonal, disturbances will be significantly associated with energy availabilities below 25kcal/kgLBM/day, regardless of training phase.

In energy balanced participants (above 25kcal/kgLBM/day):

2) The 8-week abruptly increasing running program will shorten the luteal phase length to less than or equal to 8 days in the first exercising menstrual cycle and resume a 10 to 16 day luteal phase length by the second exercising menstrual cycle. Pre-menstrual symptoms will decrease over the 8-weeks and ovulation status will remain constant.
3) Luteal phase progesterone levels will decrease from the second "control" menstrual cycle to the first "training program" menstrual cycle. Follicular phase progesterone levels will remain constant from the "control" to the "training program" menstrual cycle. Luteal phase and follicular phase estrogen levels will remain constant from the "control" to the "training program" menstrual cycles.

4) The training parameters of km-per-workout and intensity will show a non-significant association with menstrual and ovulatory disturbances.

1.5 Limitations and Delimitations

A. Participants

1) Menstrual cycle outcomes can not be extended to untrained or competitively trained individuals.

2) Menstrual cycle outcomes can not be extended to women of gynecological ages less than 12 years or greater than 27 years.

B. Measures

1) On average, the first date of elevated basal body temperature is 24 to 48 hours later than the date of the LH surge (Prior, Vigna, Schechter, et al., 1990).
2) The shift in quantitative basal temperature is an indirect measure of progesterone action.
3) Participants' recording variability will affect the accuracy of results.
4) The calculation of energy availabilities is limited in accuracy, as it is based on dietary records and estimated exercise energy expenditure over a 3-day period.

C. Methods

1) An 8-week training program will not provide information concerning the longer- term effect(s) of running training on menstrual cycle and ovulatory disturbances.
2) The independent variable of rate of running program increase will be confounded by caloric intake levels, the total volume of km run, km run per workout, as well as other training parameters.

Chapter II Review of Literature

2.1 Introduction

Menstrual cycle disturbances exist as a continuum, ranging from the cross-sectional reports of the extremes - amenorrhea and oligomenorrhea - to the prospective research revealing the more subtle changes of anovulation, shortened luteal phase, inadequate luteal phase progesterone secretion, and decreased pre-menstrual symptoms. Researchers agree that a substantial prevalence of menstrual cycle and ovulatory disturbances occur in physically active women; however, inconsistencies exist in identifying the causal factor(s). Specifically, researchers have explored two main theories: the energy availability and the stress of exercise. Stress is perceived in the hypothalamus as an increase in corticotrophin releasing hormone (CRH), resulting in a down-regulation of gonadotropin-releasing hormone, GnRH. The following review will depict the prevalence, as well as, the adaptation progressions and reversibility of menstrual cycle and ovulatory disturbances in physically active women; examine the associated negative health consequences; and explore the supporting literature and the postulated mechanisms of energy availability versus exercise stress.

2.1a Prevalence

The lengthening of the menstrual cycle and/or amenorrhea has yet to be established in prospective investigations in physically active woman. Torstveit and Sundgot-Borgen (2005) cross-sectional menstrual cycle questionnaire study revealed that elite Norwegian athletes had a similar percentage of present menstrual cycle dysfunction (16.5%) as compared to non-athletic population-based controls (15.2%). Present menstrual dysfunction included primary amenorrhea, secondary amenorrhea, oligomenorrhea, and short cycles (defined as a menstrual cycle less than 22 days). However, further analysis revealed that elite athletes competing in leanness sports (endurance, aesthetic, weight-class, and anti-gravitation sports) reported a higher history of primary amenorrhea and secondary amenorrhea in comparison to athletes in non-leanness sports (technical, ball games, and power sports) and controls (Torstveit and Sundgot-Borgen, 2005). Congruently, a review of cross-sectional in convenience sampled studies reveals a prevalence of amenorrhea between 5 and 46 % in runners and 37 and 44% in ballet dancers (DeSouza, et al. 1998). Moreover, DeSouza (2003) also documented a high prevalence of amenorrhea in aesthetic sports: primarily, gymnasts, cyclists, swimmers, and body builders. The large variability in the literature is mainly due to inconsistent classification of amenorrhea - definitions vary from 1-missed menstrual cycle in 10 months to consecutively missing menstrual

cycles for greater than or equal to 6 months (DeSouza and Metzger, 1991). This latter definition will be used.

Subtle menstrual cycle and ovulatory disturbances, including inadequate luteal phase progesterone production, shortened luteal phase, and anovulation, have been prospectively documented in physically active women. As can be viewed in Table 2.1, the reviewed prospective studies in recreationally active women reveal that between 19.7% to 55%, and an average of 37.1%, of active women maintain a regular ovulatory menstrual cycle; whereas, 45 to 80.3 %, and an average of 62.7%, of active women will experience subtle menstrual cycle disturbances (DeSouza et al., 1998; Prior, Vigna, Schechter et al., 1990; Bullen et al., 1991; Prior, Cameron, et al., 1982; and Williams et al., 1999). Further analysis of the subtle menstrual cycle disturbances in physically active women, as can be viewed in Table 2.2, illustrates that 8 to 16 % experienced luteal phase progesterone deficiencies; 21 to 66% experienced shortened luteal phase lengths; and 0 % to 42% were anovulatory (DeSouza et al., 1998; Prior, Vigna, Schechter, et al., 1990; Bullen et al., 1985; Beitins et al., 1991; Prior, Cameron et al., 1982; and Williams et al., 1999). The varying range of menstrual cycle and ovulatory disturbances is a result of the studies' contrasting amounts of exercise stress and energy deficits; the range in gynecological ages; the methods used to determine menstrual cycle status; the number of observed menstrual cycles; as well as, other uncontrollable variables such as additional stresses. In addition to the high prevalence of subtle disturbances, physically active women also display a more inconsistent menstrual cycle status as compared to sedentary controls (DeSouza et al., 1998; and Prior, J.C., Yuen, B.H., Clement, P., Bowie, L., & Thomas, J, 1982). Specifically, DeSouza et al. (1998) demonstrated that 91% of the sedentary controls had consistent ovulatory cycles over a period of three months; whereas only 54% of exercising women maintained either a consistent luteal phase deficient or anovulatory menstrual cycles. Forty-six percent of the exercising woman had inconsistent menstrual cycle status from cycle to cycle.

The reviewed prospective studies in physically active women have also documented the lengths of menstrual cycles, as well as follicular and luteal phase lengths. As can be viewed in Table 2.3, the range of the menstrual cycle lengths is between 26.8 (1.3) and 28.2 (2.6) days, averaging (+/- SEM) 27.6 +/- 1.7 days; the follicular phase lengths range between 14.4 (1.2) and 18.2 days, averaging 16.1 days; the luteal phase lengths vary between 10.13 (1.9) and 12.8 (0.7) days, averaging 11.52 days; and the luteal phase index was approximately 0.36 to 0.48, averaging a ratio of 0.41. The above menstrual cycle parameter lengths represent healthy physically active women with an average gynecological age of 16.6 (1.2) years who reported

regular menstrual cycle lengths prior to enrollment in the study (DeSouza et al., 1998; Prior, Vigna, Schechter, et al., 1990; Rogol et al., 1992; and Bonen, 1992). Moreover, the reviewed studies reflect varying volumes of exercise and energy deficits; methods used to determine menstrual cycle status; length of observations; as well as other uncontrollable variables such as outside stresses.

2.1b Menstrual Cycle Adaptations and Reversibility

Williams, Caston-Balderrama, et al. (2001) prospectively documented the development of amenorrhea in 8 cynomolgus monkeys over a period of 7 to 24 months, averaging 14.3 +/- 2.2 months. The subjects exhibited insignificant menstrual cycle disturbances up until the 2 menstrual cycles immediately preceding amenorrhea. In these two menstrual cycles, the subjects significantly increased the length of the menstrual cycle from 31+/-2 in the sedentary phase to 37+/-3 days in the cycle preceding amenorrhea; 37.5% became anovulatory; and the ovulatory menstrual cycles experienced a significant lengthening of the follicular phase from 14+/-1 days in the sedentary phase to 23+/-2 days in the cycle preceding amenorrhea. The 2-menstrual cycle abrupt transition is summarized by Williams as follows:

1) Suppression in Gonadotropin Secretion: Decrease of late luteal phase follicular stimulating hormone (FSH) secretions, which is important for the recruitment of developing follicles at the beginning of each cycle.

2) Lengthening of time needed for the development of a dominant follicle and the impairment of follicular development: An increase in menstrual cycle length, due to an increase in follicular phase length with no change in luteal phase length. Also, a significant decrease in plasma luteinizing hormone (LH) concentrations in the early follicular phase.

3) Impairment of corpus luteum secretory function: Average and peak luteal phase plasma concentrations of progesterone were reduced immediately preceding the development of amenorrhea.

4) Complete cessation of menstrual cycle with low circulating levels of gonadotropins and gonadal steroids.

The progression of menstrual cycle and ovulatory disturbances with increasing exercise has yet to be prospectively documented in physically active women. However, the time course appears to proceed from decreases in pre-menstrual symptoms, FSH, and LH pulse frequency, as well as an increase in LH pulse amplitude, to inadequate amounts of progesterone secretion, shortened luteal phase length with an increase in follicular phase length, loss of LH surge or anovulation, and eventually leading to oligomenorrhea and amenorrhea (Petit & Prior, 2000; DeSouza et al., 1998; Loucks et al. 1998; Loucks & Verdun, 1998; and Loucks & Thuma, 2003).

Moreover, the menstrual cycle and ovulatory disturbances are reversible (Prior, HoYuen, et al., 1982; Bullen et al., 1985; and Williams, Helmreich, Parfitt, Caston-Balderrama, & Cameron, 2001). Prior, HoYuen et al. (1982) prospectively documented the reversal of menstrual cycle disturbances in two marathon runners: one participant regained a normal luteal length cycle immediately following a decrease in training volume and the other participant became pregnant 6-weeks post-exercise cessation. In Bullen's et al. (1985) strenuous exercise study, 28 university students regained normal ovulatory function in 6-months after decreasing activity levels. Williams, Helmreich, et al. (2001) reversed the menstrual cycle status in four amenorrheic cynomolgus monkeys by maintaining exercising volumes and increasing caloric intake: suggesting that the increase in caloric intake alone, independently of decreased training volumes, can reverse the menstrual cycle disturbances. Conversely, Loucks & Verdun (1998) failed to reverse the disrupted LH pulsatility in exercising women with an aggressive 24-hour refeed. Regardless of the mechanisms, reduced exercise energy expenditure and/or increased caloric intake, the menstrual cycle disturbances associated with exercise are reversible.

2.1c Health

The imbalance of progesterone and estrogen characterizing menstrual cycle and ovulatory disturbances increases an active woman's health risks, primarily low cancellous bone density and infertility (Prior, 1997). Low cancellous bone mineral density (BMD) in physically active women has been controversially associated with low amounts of progesterone (Prior, Vigna, Schechter et al., 1990; and Petit, Prior, & Barr, 1999), hypoestrogenism (DeSouza, Miller, Squenzia, Luciano, Ulreich, Stier, Prestwood, & Lasley, 1997) and inadequate nutritional intake (Ihle & Loucks, 2004). Prior, Vigna, Schechter et al. (1990) research in 66 women demonstrated that the mean changes in bone density vary depending on the menstrual cycle ovulatory characteristics. Specifically, women who experienced more than one shortened luteal phase or one or more anovulatory cycles lost significant amounts of cancellous bone density by

quantitative computed tomography (QCT) per year, averaging around 4 percent per year. The study also showed a high correlation between the luteal phase index and one year changes in bone density, $r = 0.535$, $p < 0.001$ (Prior, Vigna, Schechter et al., 1990). Additionally, Prior, Vigna, Barr, & Lentle (1994) investigated the effects of cyclic medroxyprogesterone (10mg days 16-27) on spinal bone density changes in DXA and DXA-equivalent units in 61 physically active women who were stratified into therapy by initial abnormal menstrual cycle or ovulatory characteristics. The randomized double-blind placebo controlled trial compared four groups (Group A: medroxyprogesterone and 1000mg calcium; Group B: medroxyprogesterone alone; Group C: calcium alone; and Group D: calcium and medroxyprogesterone placebos) over the duration of 1-year. The net bone density increase was 2.2 +/-0.6% ($p = 0.003$) in group A, a non-significant increase of 1.2 +/-0.9% ($p=0.203$) in group B, no bone density change of -0.70 +/-0.6% ($p=0.208$) in group C, and a loss of 2.0 +/-0.6% loss of bone density ($p=0.005$) in group D. Spinal bone density was significantly increased by the cyclic medroxyprogesterone treatment (ANOVA, $p = 0.0001$), as determined by dual energy techniques. Prior (2002) concludes that the primary health consequence resulting from inadequate progesterone production, in combination with unaltered levels of estrogen, is accelerated bone loss.

DeSouza (2003) concurs that BMD may be affected in recreationally active women; however, she postulates that chronic estrogen deficient cycles are the primary factors affecting bone health. DeSouza's et al. (1997) 3-month cross-sectional study compared dual x-ray absorptiometry (DXA) bone mineral densities between sedentary (SedOvul: n=9) and exercising (ExOvul: n=14) ovulatory participants, as well as, exercising participants with luteal phase deficiencies (ExLPD: n=10). The ExLPD significantly lower luteal phase progesterone ($p=0.0004$) did not result in any bone mineral density differences as compared to the SedOvul and ExOvul groups with normal levels of progesterone. Furthermore, the three groups displayed comparable estrogen levels; excluding the early follicular phase, where the exercising groups had lower estrogen levels ($p=0.043$). Although a non-significant correlation was found between the early follicular phase estrogen levels and bone mineral density ($p=0.06$), DeSouza postulated that estradiol is the most important hormone for maintaining bone mass in women with subtle menstrual cycle disturbances. In relation to the research of Prior, Vigna, Schechter et al. (1990), DeSouza (2003) asserts that the contrasting findings are mainly due to the "confusing effect of combining estrogen deficient anovulatory cycles with LPD cycles, as it is well established that anovulatory estrogen cycles may affect bone health."(pg 1554). However, upon further analysis of Prior, Vigna, Schechter et al., (1990) 1-year prospective study, Petit and colleagues (1999)

revealed that the bone mineral density of forty-two runners was significantly related to luteal length ($r=0.481$, $p = 0.001$) and average progesterone ($r=0.381$, $p=0.015$) but not to estrogen ($r=0.211$, $p=0.198$). As such, the luteal phase length and amount of progesterone appears to act positively on cancellous bone in healthy women with normal estrogen levels. Ihle & Loucks (2004) further note that the role of hypoestrogenism must be reconsidered, as estrogen supplementation trials have not fully reversed the bone loss.

Petit et al. (1999) also postulated a potentially important alternative mechanism, namely high dietary restraint and low energy intakes, as BMD was correlated to the average energy intake ($r=0.746$, $p=0.0008$) in the twelve consistently normally ovulatory women. Ihle & Loucks (2004) explored the energy intake correlation by investigating energy availability in relationship to indirect markers of bone formation markers (OC: osteocalcin and PICP: serum type I procollagen carboxy-terminal propeptide), as well as, the bone resorption marker (NTX: N-terminal telopeptide) in physically active women. Participants expended 15 kilocalories per kilogram of lean body mass in exercise per day (kcal/kgLBM/day), in conjunction with balanced (45kcal/kgLBM/day) and restricted (10, 20, 30kcal/kgLBM/day) energy availability treatments. A dose-response relationship was demonstrated as OC concentrations deceased by 28% ($p=0.0001$), 32% ($p=0.002$), and 11% ($p=0.02$) with the restricted energy availability treatments of 10, 20, and 30kcal/kgLBM/day, respectively. PICP also declined linearly with energy availability ($p< 10^{-6}$). By contrast, NTX concentrations were raised by 34% ($p<0.001$) at a restriction of 10kcal/kgLBM/day; whereas, the treatments at 20 and 30kcal/kgLBM/day had no effect ($p> 0.4$). Similarly to NTX, estradiol was only altered at 10kcal/kgLBM/day energy restriction. Ihle & Loucks conclude that a severe energy restriction will suppress estradiol, increase bone resorption, and become uncoupled from suppressed bone formation within 5-days; resulting in potentially irreversible bone mineral density reduction. A comparison between the bone mineral density studies is limited due to differing lengths of observation, methodologies, and outcome variables. Future bone resorption and formation research is necessary in order to understand the underlying effects of progesterone and estrogen levels and dietary intakes (Prior, 1996b).

Infertility is another health concern resulting from anovulatory cycles. Additionally, the effects of shortened luteal phases and inadequate amounts of progesterone may also affect fertility in relation to the implantation and early maintenance of a conceptus. However, the correlated relationship between progesterone levels and fertility remains speculative, as research is limited in this area (Williams, Caston-Balderrama, et al., 2001). It is of note that menstrual

cycles not secreting any progesterone indicate that an egg has not been released and the cycle is infertile. In addition to bone and fertility consequences, women experiencing higher amounts of estrogen, lowered progesterone levels, as well as an increase in LH have also been found to potentially experience increased menstrual flow, troubling premenstrual symptoms, irregular and heavy uterine bleeding, anemia, chronic endometrial hyperplasia and cancer, breast cancer, as well as acne and hirsutism (Prior, 1997). The resulting health consequences of menstrual cycle abnormalities can be prevented in physically active women by identifying the causal factor(s), namely energy availability and/or exercise stress.

2.2 Dichotomy of theories: Energy Availability versus Exercise Stress

2.2a Energy Availability:

To date, research concludes that a woman's reproductive system depends on energy availability; that is the difference between dietary intake and exercise energy expenditure, not the stress of exercise. Precisely, Loucks has explicitly demonstrated through a series of investigations that short-term energy availability below 19.6+/- 0.1kcal/kgLBM/day over a four to five day period in exercising women decreases LH pulse frequency and increases LH pulse amplitude; Williams, Caston-Balderrama, et al. (2001) documented the development of amenorrhea associated with an energy deficit in 8 exercising cynomolgus monkeys over an average period of 14.3 +/- 2.2 months; and DeSouza et al. (1998) prospectively observed menstrual cycle and ovulatory disturbances associated energy deficits between sedentary ovulatory, exercising ovulatory, exercising luteal phase deficit, and exercising anovulatory participants. Although a relationship is evident between an energy availability below DeSouza's finding of 24.7kcal/kgLBM/day and menstrual cycle disturbances; a detailed review of the energy availability findings above 25kcal/kgLBM/day reveal subtle and noteworthy disagreements (DeSouza et al., 1998; Loucks et al., 1998, Loucks & Verdun, 1998; and Loucks & Thuma, 2003). It is plausible that the discrepancies are reflective of methodological differences: Loucks' short-term experiment in comparison to DeSouza's 3-month observations; or Loucks' outcome variables of LH pulse frequency and amplitude versus DeSouza's documentation of luteal phase and ovulation status. It is additionally plausible that the discrepancies potentially reveal alternate mechanism(s), independent of an energy availability threshold; namely the chronic accumulation of moderate energy deficiencies, daily exercise stresses, and/or a combination of mechanisms.

Loucks & Heath (1994) first demonstrated the link between restricted energy intake and the disturbances of reproductive cycle rhythms in healthy women with an average gynecological age of 7.7 +/-1.2 years. The seven eligible participants had at least 3 months of documented 26 and 32 days in length menstrual cycles; exercised less than 60 minutes of habitual aerobic activity per week during the past 3 months; and presented estimated habitual energy intakes between 35 and 55kcal/kgLBM/day. Two liquid clinical dietary treatments, a balanced dietary intake of 45kcal/kgLBM/day and a restricted dietary intake of 10kcal/kgLBM/day, were prospectively controlled over 4 days. The treatments were assigned in random order in the follicular phases of two discrete menstrual cycles, which were separated by at least two menstrual cycles. Blood was drawn for three consecutive days 2-days prior to the treatment, as well as, 24-hours post treatment. The participants' regular sedentary activity levels were calculated automatically by activity monitors. Over the 4-day period, the dietary treatments proved significantly different in terms of dietary intakes (Restricted: 9.9 +/-0.1 versus Balanced: 43.4 +/-1.0kcal/kgLBM/day, $p<10^{-7}$), energy balance (Restricted: -33.2 +/- 0.8 versus Balanced: -0.8 +/- 1.6kcal/kgLBM/day, $p < 10^{-6}$), and weight loss (Restricted: -1.5kg). Moreover, the low energy availability treatment decreased luteinizing hormone pulse frequency by 23% ($p< 0.01$) during the day and increased luteinizing pulse amplitude by 40% ($p< 0.05$) during the night, as compared to the balanced 45kcal/kgLBM/day intakes. The 24-h transverse means of LH ($p=0.3$), FSH ($p= 0.2$), and E_2 ($p=0.3$) were unaffected by the low energy availability.

Subsequently, Loucks et al. (1998) attempted to differentiate whether low energy availability or exercise stress disrupted LH pulsatility by controlling both dietary intake and exercise-expended energy. Energy availability was defined as dietary intake minus exercise energy expenditure and exercise stress was operationally defined as everything associated with exercise except its energy cost. Accordingly, nine participants with an average gynecological age of 8.7 +/- 1.1 were assigned in random order to two 4-day dietary intakes of either 75 or 45 kcal/kgLBM/day; while concurrently expending 30kcal/kgLBM/day by walking on a graded treadmill in sequences of 30-min bouts at 70% of the aerobic capacity, interrupted by 10 min rest periods. The energy treatments proved significantly different in terms energy availability (Restricted: 12.7 +/- 1.1 versus Balanced: 48 +/- 0.8kcal/kgLBM/day, $p < 10^{-9}$) and weight loss (Restricted: -1.7 +/-0.2 versus Balanced: 0.1+/-0.2 kg, $p < 0.0001$). Noteworthy, exercise energy expenditures between groups were not significantly different ($p = 0.9$). The low energy availability decreased LH pulse frequency by 10% during waking hours and increased LH pulse amplitude by 36% during sleeping hours, as compared to the balanced energy availability. The

24-hour LH transverse mean ($p>0.05$) and E_2 ($p = 0.6$) remain unaffected; however, 24-hour mean FSH levels were significantly raised ($p = 0.04$) by the low energy availability.

In comparison to Loucks & Heath (1994), the LH pulsatility effects of the dietary restriction in combination with exercise energy expenditure (Loucks et al., 1998) were significantly smaller than those of dietary restriction alone. Specifically, LH pulse frequency caused by the exercise energy expenditure (↓ 10%) was reported as 60% smaller than the effect of low energy availability caused by dietary restriction alone (↓ 23%), $p< 0.03$; the LH pulse amplitude increases were similar between the two energy availability treatments, $p < 0.5$; and FSH was significantly raised in the exercising energy-expenditure treatment ($p=0.04$) in comparison to a non-significant change in the dietary-restriction-only treatment ($p=0.2$). The difference in the decreased LH pulse frequency and increase in FSH excretion with the exercise energy expenditure is unknown; however, Loucks & Redman (2004) hypothesize that exercise played a "protective" role with respect to reproduction function. Specifically, the energy restricted exercising women "had higher carbohydrate availability, due to a glucose sparing alteration in skeletal muscle fuel selection" (pg 5). Nonetheless, based on the study's (Loucks et al., 1998) results and in comparison to Loucks' & Heath's (1994) findings, Loucks concluded that 4-days of intense exercise has "no disruptive effect on LH pulsatility apart from the impact of its energy cost on energy availability and that exercise energy expenditure disturbs LH pulsatility less than the equivalent amount of dietary energy restriction" (pg 43-44).

In an effort to substantiate the key role of low energy availability, Loucks & Verdun (1998) investigated the LH pulsatility effects of refeeding eight energetically deficient women with an average gynecological age of 7.8 +/-1.0 years. The participants completed 15 kcal/kgLBM of treadmill walking per day, while concurrently consuming 25kcal/kgLBM/day over a 5-day period followed by an aggressive refeed of 90kcal/kgLBM/day over 24-hours. Blood samples were collected for 24-hours on the 5^{th} day of low energy availability treatment and then for 24 hours during the refeeding. The restricted energy availability phase resulted in a significant loss of 2.4 +/- 0.2 kg of body weight; decreased LH pulse frequency by 57 %, $p<10^{-4}$; and increase LH pulse amplitude by 94%, $p<10^{-4}$. The 24-h aggressive refeeding protocol proved to be inadequate in re-stimulating the disrupted reproductive system, as LH pulse frequency levels did not raise above the fifth percentile and LH pulse amplitude remained completely unaffected, $p > 0.9$. Furthermore, the low energy availability and aggressive refeed had no significant effects on estradiol (E_2).

Loucks completed the series of low energy availability investigations by successfully quantifying the dependence of LH pulsatility on energy availability in healthy, regularly menstruating, habitually sedentary women with an average gynecological age of 8.5 +/-1.0 years. Following similar eligibility criteria and methodological procedures as previously described, Loucks & Thuma (2003) manipulated the incremental effects of balanced (45kcal/kgLBM/day) and restricted (10, 20, 30kcal/kgLBM/day) treatments over a 5-day period, by controlling both dietary intake and exercise energy expenditure. Following Loucks & Verdun's (1998) identical exercise protocol, participants performed 15kcal/kgLBM/day of walking on a motorized treadmill. The restricted energy available treatments 10, 20, and 30kcal/kgLBM/day significantly decreased the participants' body weights; the restricted energy availability treatments of 10 and 20kcal/kgLBM suppressed LH pulse frequency and increased LH pulse amplitude, $p < 0.04$; the restricted energy availability of 10kcal/kgLBM suppressed E_2 concentrations by 15%, $p < 0.01$; and the 24-h mean FSH concentrations remained unchanged, $p > 0.4$. As can be viewed in Table 2.4 and Figure 2.1, Loucks & Thuma's (2003) results in combination with Loucks' previous experiments explicitly demonstrate that LH pulsatility is disrupted abruptly at a threshold of energy availability no higher than 30kcal/kgLBM/day. Table 2.4 provides a detailed summary of the LH pulsatility effects by separating Loucks' results by study and by group. Accordingly, Figure 2.1 illustrates the energy availabilities that proved to non-significantly affect LH pulsatility - squares (B45, 1998[a]; R30, 2003; B45, 2003) versus the energy availability groups significantly affecting LH pulsatility - triangles (R10, 1998[a]; R10, 1998[b]; R10, 2003; R20, 2003). Refer to Table 2.4 for coding details; e.g. B45, 1998[a] refers to Loucks et al., 1998 study group, which had a balanced (B) energy availability of 45kcal/kgLBM/day. Loucks & Thuma's (2003) experiment also revealed that "the disruptive effects of sub-threshold energy availability were bimodal, with substantially larger effect occurring in the subjects with the shortest luteal phases (11d); and the incremental effects of restricted energy availability most closely resembled those in glucose, B-HOB, GH, and cortisol" (pg305-306). It is of note that Loucks' findings reflect the LH pulsatility effects of severe dietary restrictions in combination with an abruptly increasing 4 to 5 day exercise regimen. Subsequently, the findings can not be extended to the more moderate energy-deficiencies behaviours typically sustained by regularly active women.

Williams, Caston-Balderrama, et al. (2001) and Williams, Helmreich, et al. (2001) concur with Louck's energy deficit work, as she prospectively documented the development and reversal of amenorrhea in association with energy availability. Sixteen regularly ovulatory

menstruating and habitually sedentary cynomolgus monkeys were divided into two groups: an exercise group (n=8) and a matched sedentary control group (n=8). The exercising subjects were gradually introduced to the treadmill and increased at their own pace to run 7 days per week for a total of 2 hours per day. The strenuous exercise program lead all eight exercising monkeys to develop amenorrhea over a period ranging from 7 to 24 months, averaging 14.3 +/- 2.2 months; while none of the eight control subjects developed amenorrhea. Amenorrhea was defined as a loss of menses for a 90-day period. Although energy availability is not well documented, the constant caloric intake, averaging 263.3+/-16 calories, in combination with increasing exercise energy expenditures is suggestive of a low energy availability status. Moreover, Williams, Helmreich, et al. (2001) observed altered hormonal markers indicative of a hypometabolic state. The dependence of the menstrual cycle on energy availability was further demonstrated by aggressively refeeding four of the "exercised-induced amenorrheic" monkeys (Williams, Helmreich, et al. 2001). During the refeed, the monkeys' caloric intakes were significantly increased by 138, 141, 163, and 181 % above their amenorrheic caloric intakes; while concurrently maintaining the identical exercise energy expenditure program of running approximately 12 km per day. The overfeeding significantly increased daily energy intake by approximately 58%; increased mean body weight by 6%; and restored LH, FSH, and E_2 values. The number of days until pre-ovulatory LH surge recovery varied between 12 and 57 days; which significantly negatively correlated with energy intake ($r = -0.98$, $p < 0.024$). Since dietary supplementation alone normalized each of the subjects' menstrual cycles, Williams, Helmreich, et al. (2001) concluded that the suppression of reproductive function is due to the energy cost associated with exercise and not other exercise factors such as physical or psychological stress.

DeSouza et al. (1998) broadens Loucks' short-term analyses and William's monkey primate model by prospectively observing physically active women over a period of 3-months. The cohort of thirty-five regularly cycling women documented their daily training activities; tracked their dietary intakes and energy expenditures over 7-days in the follicular phase of each menstrual cycle; and collected 8-hour urine samples beginning in the follicular phase until the next menses. The 3-month observation revealed that the exercising women experienced a significantly greater occurrence of luteal phase deficiencies and anovulatory menstrual cycles (58%) as compared to the sedentary women (9 %), $p < 0.006$. Based on the menstrual cycle results, the cohort was divided into four groups: 1) sedentary ovulatory (SedOvul), $n = 11$; 2) exercising ovulatory (ExOvul), $n = 10$; 3) exercising luteal phase deficiency (ExLPD), $n = 10$; 4) exercising anovulatory (ExAnov), $n = 4$. Hormonally, ExOvul, ExLPD, and ExAnov

demonstrated a significant suppression of estradiol excretion during early follicular phase as compared to SedOvul. Moreover, ExAnov excreted a significantly less E1C over the follicular and luteal phase than SedOvul, ExOvul, or ExLPD ($p<0.05$). ExAnov and ExLPD excreted significantly less PdG than SedOvul and ExOvul. Furthermore, ExOvul demonstrated a lower luteal phase PdG level than SedOvul ($p<0.05$). ExLPD excreted significantly less FSH than SedOvul and ExOvul ($p<0.007$); whereas, ExAnov excreted a significantly greater amount than SedOvul, ExOvul, and ExLPD. There were no significant associations between menstrual cycle status and the varying exercise expenditures: SedOvul expended significantly less exercise energy (0kcal/kgLBM/day) as compared to the ExOvul (10.14kcal/kgLBM/day), ExLPD (10.45 kcal/kgLBM/day), and ExAnov (5.75 kcal/kgLBM/day). Conversely, significant differences were noted in energy intakes, total energy expenditures, energy availability, and energy balance.

In congruency with Loucks' and Williams' low energy availability findings, DeSouza's ExAnov menstrual cycles consumed significantly lower dietary intakes ($p=0.03$), as well as, displayed substantially lower energy availabilities ($p< 0.001$) and energy balances ($p=0.001$) as compared to all other groups. Moreover, the ExAnov group's energy availability of 24.7 kcal/kgLBM/day corresponds to Loucks' energy availability threshold of below 30 kcal/kgLBM/day. Conversely, DeSouza's ExOvul and ExLPD groups present conflicting results with Loucks & Thuma's (2003) findings that LH pusatility can be maintained with a 33% restriction of energy availability to 30 kcal/kgLBM/day. Precisely, the exercising group with luteal phase deficiencies displayed an energy availability of 2.2 kcal/kgLBM/day above the threshold at 32.2 kcal/kgLBM/day; whereas, the exercising group that maintained regular ovulatory function presented a 1.3 kcal/kgLBM/day below Loucks' threshold. The conflicting results are further demonstrated by comparing the ExLPD group to Loucks' energy balanced and availability groups, which did not significantly affect LH pulsatility (view Figures 2.2 and 2.3). In the energy balanced (EB)/energy availability (EA) figures, the dotted line represents the EB/EA threshold below which menstrual cycle disturbances occur; the line with squares represents the EB/EA values that did not disturb the menstrual cycles; and the black triangle displays DeSouza's ExLPD outlier group. In the EB/EA graphs, Loucks & Thuma's (2003) restricted EB of -13.9 kcal/kgLBM/day or an EA of 29.7kcal/kgLBM/day did not adversely affect LH pulsatility; whereas, DeSouza's ExLPD group's EB of -0.82 kcal/kgLBM/day or EA of 32.2 kcal/kgLBM/day characterized the luteal deficient menstrual cycles. Additionally, the intra-study comparison reveals that the ExOvul group exhibited lower energy availabilities than the ExLPD group by 3.5kcal/kgLBM/day (DeSouza et al., 1998). As can be viewed in the

Figure 2.4, the ExOvul group's energy balance of -5.03kcal/kgLBM/day proved significantly lower as compared to the SedOvul group's energy balance of 1 kcal/kgLBM/day (squares); whereas, the ExLPD group's energy balance of -0.82 kcal/kgLBM/day (triangle) was non-significantly different.

It is plausible that the inter-study incongruencies may be negligible or attributable to the dissimilar outcome variables, length of observations, and methodologies. In reference to outcome variables, it is possible that LH pulsatility responds to a different threshold than does luteal function. Precisely, LH pulsatility is not disrupted above an energy balance threshold of -13.9 kcal/kgLBM/day or an energy availability threshold of 29.7 kcal/kgLBM/day. Conversely, the luteal phase inadequacies can potentially occur around an energy balance of -0.82kcal/kgLBM/day or an energy availability of 32.2kcal/kgLBM/day. However, it remains that DeSouza's ExOvul group was able to maintain regular luteal function at a lower energy balance of -5.03 kcal/kgLBM/day or energy availability of 28.7kcal/kgLBM/day in comparison to the ExLPD group. In relation to the length of observation, it is plausible that DeSouza's 3-months observations are more reflective of the chronic effects of a training regimen; and in turn, more reflective of the mechanism in recreationally active women. Potentially, Loucks' severe energy restrictions had to be greater because of the short, 4 to 5 days, time period. In respect to methodologies, it is possible that Loucks' findings are more precise due to the randomized repeated measure design versus DeSouza's cross-sectional study. Furthermore, Loucks calculated energy availabilities based on continuous measurements over the entire duration of the study versus DeSouza's estimated energy availabilities based on monthly 7-day dietary records and energy expenditure calculations. Similarly, the intra-study comparison of DeSouza's groups may be limited by the cross-sectional nature of the study, as well as the potentially inaccurate measurements of energy intake and expenditure. However, it is also possible that the conflict between the ExLPD's group and the other non-significant groups (squares in Figures 2.2 and 2.3) is attributable to the existence of an alternative mechanism.

Williams (2003) postulates that energy availability may not be the only mechanism; as emotional or psychological stresses associated with daily physical training, not the physical stress of exercise per se, can potentially contribute to the menstrual cycle disturbances. Williams (2003) further hypothesizes "the effects of challenges to the reproductive axis, whether psychological or metabolic in nature, may in part depend on an individual's own inherent susceptibility to menstrual dysfunction". Moreover, "possible indications of the 'robustness,' e.g. resistance to menstrual disturbances caused by environmental factors, are gynecological age,

the presence or absence of previous menstrual disturbances, and one's responsiveness to life stressors" (pg1569). It is plausible that the ExLPD group was inherently more susceptible; however, such factors as life stressors were not documented and the reproductive characteristics were similar between DeSouza's groups, as well as in relation to Loucks' groups. Another possible mechanism is that "larger disruptions of LH pulsatility and ovarian function in athletes might derive from their chronic maintenance of low energy availability habits...more moderate energy-deficiencies behaviors are sustained for periods of weeks, months, and years" (Loucks et al., 1998; pg44). Lastly, it is plausible that the accumulation of daily repetitive exercise stresses resulted in the menstrual cycle and ovulatory disturbances.

2.2b Exercise Stress

Previous research has demonstrated an association between menstrual cycle disturbances and the training variables of total volume, distance run, intensity, and competitive stress. (Prior, Vigna, Schechter, et al., 1990; Shangold, Freedman, Thyssen, & Mengold, 1979; Prior, HoYuen, et al., 1982; Prior, Cameron, HoYuen, & Thomas, 1982; Bullen et al., 1985; Rogol et al., 1992; Seyle, 1939; Williams et al., 1999). However, investigators have yet to establish a causal relationship. An inter-study contrast of training volumes per workout/day, per week/cycle phase, and per menstrual cycle, as well as, intensities demonstrates the conflicting results in relation to menstrual cycle and ovulatory disturbances, as well as, reveals the existing limitations of exercise stress research; mainly low numbers of participants, imprecise measuring techniques, and the lack of control groups. Additionally, the comparisons highlight the investigators' neglect of key confounding variables, namely energy availability, as well as the unexplored rate of training program increases.

Prior, HoYuen, et al. (1982), Prior, Cameron, et al. (1982) and Shangold, et al. (1979) explored menstrual cycle disturbances in relation to distances run per day/workout. Prior, HoYuen, et al. (1982) research in 2 gynecologically mature marathoner runners showed one runner experiencing 11 menstrual cycles with a less than 9-day luteal phase, while completing between 8 and 13 km per day throughout 12 menstrual cycles. The other runner experienced 6 out of 12 shortened luteal phase cycles, while running between 4.8 and 10.5 km per day. Similarly, Shangold et al. (1979) presented a chronic long distance runner experiencing a less than 9-day luteal phase in accordance with running volumes greater than 56 km per week or workouts equalling around 9.6 km. Shangold et al. (1979) attempted to define the relationship more precisely by revealing that the chronic runner's luteal phase length was inversely related to

the total distance run during the first 7 days of the follicular phase, as well as to the average weekly km run during the luteal phase. Both studies' limited sample sizes in conjunction with Shangold's imprecise luteal length measuring techniques of cervical mucous changes restrict the inferences concerning the training volumes.

Correspondingly, the Prior, Cameron, et al. (1982) 14-runner study demonstrated a correlation between the number of km run per day during pre-menstrual phase and the luteal phase length ($r = -0.41$, $p < 0.01$). Additionally, the luteal phase lengths, averaging approximately 6.4 +/- 0.9 days, were found to be more strongly correlated ($r = -0.52$, $p < 0.01$) with their average run length per training run (total km run per cycle / # of running days) of approximately 15.6 +/- 3.12 km per training run (Prior, Cameron, et al., 1982). The potential relationship existing between ovulation disturbances and a specific training volume per day/workout remains inconclusive, as ovulatory disturbances have been documented with training volumes as low as 4.8 km of running per day and as high as 15.6 km per workout. Furthermore, training bouts equalling 9.6 km per day have resulted in both unchanged menstrual cycle rhythm (Bonen, 1992) and ovulatory disturbances (Shangold et al., 1979).

The contrasting training program volumes in Bullen et al. (1985), 80 km in seven days, in comparison to Williams' et al. (1999), 78 to107 km in 10 to 20 days, potentially reveals "a dose response relationship between the volume of exercise performed and the severity of reproductive disturbances observed" (Williams et al., 1999; pg.956). Specifically, the combined luteal phase results of William's et al. (1999) groups that either trained solely in the follicular or the luteal phase corroborated with Bullen's et al. (1985) weight-maintenance group findings: Bullen's training program resulted in 8 out of 12 participants experiencing an abnormal luteal function; whereas, William's training program resulted in 5 out of 9 runners experienced shortened luteal phases. However, Bullen's more demanding exercise energy expenditure program resulted in an additional 5 out of 12 weight-maintenance participants to experience anovulatory cycles. Although William's dose relationship seems logical, the comparison is confounded by the identical rates of training program increases and undocumented energy availabilities. Furthermore, Rogol's et al. (1992) 1-year gradually increasing training program with similar training volumes to Bullen's, 64-104km per week, did not disrupt the participants' menstrual cycle rhythms. Moreover, Prior, Vigna, Schechter, et al. (1990) 66-women study, of whom 19 runners trained for and competed in a marathon, also did not support William's suggestion of an inversely related dose response. Specifically, the three running groups of less than 4.7 +/- 7.9km per menstrual cycle, 54.2 +/- 17.4km per menstrual cycle, or 142.2 +/- 60.0 km per menstrual

cycle did not reveal any significant subtle menstrual cycle disturbance differences. However, the combination of regular runners with the marathon runners (54.2 +/- 17.4km and 142.2 +/- 60.0 km per menstrual cycle) displays a higher prevalence of shortened luteal and anovulatory phases as compared to the normally active group, although statistically non-significant (refer to Table 2.5). In addition to Prior, Vigna, Schechter, et al. (1990) findings, Bonen (1992) also confirmed that participants were able to maintain training volumes between 49 and 153 km per cycle without any disturbances to the menstrual cycle. A further in-depth comparison between Bullen's et al. (1985), Williams' et al. (1999), Rogol's et al. (1992), and Bonen's (1992) confounders and limitations will be discussed below.

In addition to the indistinguishable relationship with total volumes, the level of intensity also appears to have minimal, if any effect on menstrual cycle rhythms. The reported intensities of approximately 70 to 80% of VO_2max, above 80% of max heart rate, or above lactate threshold appear to have minimal effect as each have been associated with varying menstrual cycle outcomes. Rogol et al. (1992) did reveal a statistically significant shortening of luteal phase length when exercising above lactate threshold, as compared to at lactate threshold. However, the luteal phase length still remained within the healthy range. As can be viewed in Table 2.6, the menstrual cycle also appears to be robust to the other parameters such as number of training sessions per week, duration of program, type of training, and initial training status. The unsuccessful determination of a consistent relationship, if any exists, between the training parameters of total volume, intensities, frequency, and duration in relation to menstrual cycle disturbances is potentially a result of small sample sizes, imprecise measuring techniques, and the lack of control groups. It is also plausible that commonly neglected denominator of energy availability confounds the training parameter findings. Furthermore, contrasting the existing short- and long-term abruptly and gradually increasing, as well as maintenance training programs reveals that training parameter research may have also neglected the unexplored notion of rate of training increase.

Hans Seyle (1939) first demonstrated the link between physical activity and the disturbances of reproductive cycle rhythms, as he manipulated the rates at which rats increased training on a motorized wheel. In Seyle's study, rats were either forced to begin exercise abruptly, increased gradually, or maintained their normal activity level. The results demonstrated that the abruptly increasing activity group developed anestrus, while the progressively increasing and maintenance groups maintained apparently normal reproductive cycles by ovarian histology. Although confounding factors of weight, changes in weight, and others were not well controlled

or documented, it is speculated that the outcomes were a result of the differing rates of training increases. Since Seyle's observations, there have been no studies, animal or human, investigating this idea that rate of training increases over a sufficient period of time may affect menstrual cycle and ovulatory function. Furthermore, no prospective human studies have ever established a causal relationship between physical activity and amenorrhea.

Loucks' series of 4- to 5-day investigations and Williams, Young, McArthur, Bullen, Skrinar, and Turnbull (1995) 3-day analysis explored the notion of abruptly increasing training programs over short periods of time. As described in the "energy availability" section, Loucks abruptly increased the participants' habitual sedentary activity levels, less than 60 minutes of aerobic activity over the past 3-months, to exercising between 93+/-2 to 164+/-7 minutes per day over a 4- to 5-day period. Participants expended 15 to 30kcal/kgLBM/day by walking on a graded treadmill in sequences of 30-min bouts at 70% of the aerobic capacity, interrupted by 10 min rest period. The short-term stressful increase in walking (1400kcal/day at 70% of aerobic capacity), in which Loucks et al. (1998) relatively compared to "running more than 15 miles a day, a workload exceeding the training regimen of all but the most extreme ultramarathon competitors" (pg44), had no disruptive effects on LH pulsatility apart from the impact of its energy costs on energy availability. In agreement, Williams et al. (1995) investigated the exercise stress effects of an abruptly increasing training program over 3-days. Four athletes, with an average gynecological age of 16.8 years, completed three treatments in random order : 1) a control: participants consumed sufficient calories to maintain weight; 2) an abrupt, short-term increase in training volume (STTI): participants maintained weight and increased exercise by completing three 90-min treadmill runs at 74% VO_{2max}; and 3) a combination of caloric restriction and short term training (DIET/STTI): participants completed STTI and consumed 60% of the calories necessary to maintain weight. Blood sampling revealed a decrease in LH pulse frequency ($p<0.003$) in the DIET/STTI group only and no significant difference in overall peak amplitude between treatments. In comparing LH parameters before and after exercise, the intense exercise revealed no significant effects (Williams et al., 1995). Both Loucks' and Williams' short-term strenuous exercise findings confirmed that LH secretion was disrupted only when caloric intake was insufficient to prevent changes in energy balance. It is of note that these findings are limited to the effects of short-term intense energy deficits. As such, the results cannot be extended to the effects of chronic moderate energy deficits or daily training stress of a recreational training program.

Moreover, Williams et al. (1994) investigated the LH pulsatility effects of a short-term moderately increasing training session in sixteen participants with an average gynecological of 14.1 (1.5) years. The moderate exercise regime consisted of one-hour of progressive exercise at approximately 50% (20 minutes), 60% (20 minutes), and 70% (20 minutes) of their predetermined $VO_{2\ max.}$ Blood sampling found no suppression of LH frequency and a small but significant increase in maximum peak amplitude ($p < 0.05$) and incremental peak amplitude ($p < 0.05$). It is inconclusive if the moderate exercise, itself, or if the cost of energy influenced LH amplitude, as energy availability was not reported in this study. The contrast of the exercise protocols and reported exercise energy expenditures, moderately increasing over 60 minutes versus abrupt increases over 3 to 5 days, suggest that the LH pulsatility effects are tapered in association with decreased exercise energy expenditure and energy deficits. However, it is of note that Loucks' participants may have been more susceptible to LH pulsatility alterations, as they were, on average, 7.15 gynecological years younger than Williams' participants. Furthermore, the comparison is limited with Williams' et al. (1995) sample size of four participants and Williams et al. (1994) neglect to report exercise energy availability.

In contrast to short-term abruptly increasing programs, a high prevalence of ovulatory disturbances has been found while participants complete abruptly increasing running programs over a 2-month period (Bullen et al., 1985 and Williams et al., 1999). Bullen et al. (1985) investigated the effects of a 2-month strenuous and high volume training program by randomly assigning twenty-eight untrained university aged women to either a weight-loss ($n = 16$) or a weight-maintenance ($n = 12$) group. The high volume running program commenced at 6.4 km per day and the daily distance was increased by 2.4 km for five successive weeks, equalling an abrupt increase of 12 km or 15 to 37.5% per week. The program attained a maximum distance of 16.1 km per day by the fifth week, which was held constant thereafter. Participants were also involved in three and half hours of extra activities such as playing volleyball, cycling, and stretching. In regards to dietary control, the weight maintenance group was stabilized to a maximum day-to-day fluctuation of 2kg; whereas, the weight-loss group was limited to a 0.45kg loss per week. After the strenuous 8-week program, 14.3% (4/28) of the participants maintained a normal menstrual cycle in either one or both training months. In the weight-maintenance group, 66% experienced abnormal luteal function and 42% became anovulatory. In the weight-loss group, 63% experienced abnormal luteal phase and 81% became anovulatory. Although inconclusive, the additional restricted energy availability deficit in the weight-loss group, resulting in a higher amount of anovulatory cycles and delayed menses, appears to support

Loucks' notion of energy deficit. The mechanism in the weight-maintenance group remains speculative as energy availability, the accumulation of moderate energy deficits, and the stress of an abruptly increasing training program were not well documented; and thus confound each other. Inferences are also limited due the women's young gynecological age averaging around 10 +/- 0.6 years; the lack of a control group; and given that all women were placed in a new environment during the training. As described in the training parameter section, the effects of high training volumes and strenuous intensities appear to be negligible.

In a further attempt to unravel the mechanism, Beitins, McArthur, Turnball, Skrinar, & Bullen (1991) further investigated the 18 out of 28 women identified in Bullen's study with abnormal luteal function. The 18 participants had a total of 20 menstrual cycles with luteal phase deficiencies: thirty percent had inadequate luteal phases and seventy percent had shortened luteal phases. At the end of 8-weeks, the shortened luteal phase group lost 1.2 +/- 1.2 kg in weight, $p = 0.046$ and the inadequate luteal phase group lost 1.3 +/- 1.45 kg in weight, $p = 0.008$. In turn, Beitins et al. (1991) speculated that the weight loss, indicative of energy deficit, may have disrupted the luteal phases. However, weight-loss, itself, is not a sensitive indicator of energy availability. In the Table 2.7, a comparison of the weight loss in the short-term (3-5days) and long-term (2-months to a year) studies reveal that significant ovulatory and luteal disturbances occur around an average loss of 1.68 kg in body weight; whereas, an average loss of 0.37 kg in body weight is associated with the maintenance of menstrual cycle function. Incongruously, Loucks & Thuma (2003) restricted group of 30kcal/kgLBM/day lost 1.3 kg and Bonen's (1992) group that ran close to 32km/wk over 4-months lost 1.9 kg with no significant disturbances to menstrual cycle function. Furthermore, studies have reported decreases in energy balance (i.e. DeSouza et al., 1998; ExOvul: -3.9 +/-1.7 and ExLPD: -1.4+/-1.4kcal/kgBW/day) without any significant loss in weight (DeSouza et al., 1998: body weight did not differ, $p > 0.05$). As a result, Beitins reporting of the weight loss in the abnormal luteal phase participants is suggestive of an energy deficit but remains inconclusive. Subsequently, the role of Bullen's et al. (1985) abruptly increase training program over 8-weeks remains illusive. As Beitins et al. (1991) commented that the disrupted neuroendocrine system may be a result of "the strenuous exercise program per se, the increased caloric expenditure, or the stress hormones ..." (pg1356).

Williams et al. (1999) also demonstrated a high prevalence of luteal phase disturbances by imposing Bullen's abruptly increasing training program in either the follicular or luteal phase, independently, over 3-months. Fifteen untrained runners with an average gynecological age of 8.1 +/-0.7 years were randomly assigned to one of four groups: a) an active control group, who

were progressively conditioned to a low volume of exercise; b) a passive control group, who were not engaged in exercise training; c) a follicular phase group, who trained solely during the follicular phase; d) a luteal phase group, who trained solely during the luteal phase. Forty-five percent of the combined luteal and follicular phase participants experienced luteal phase shortenings, as compared to none of the control participants. Similar to the findings in the training parameter section, the occurrence of luteal phase defects had no correlation with the total mileage. Conversely, the women with luteal phase defects exhibited a decrease (137.5 +/- 12.5 to 126.7 +/-9.2 kilojoules/kg/day) in energy intake; whereas those who maintained normal cycles exhibited an increase (131.7 +/-9.2 to 142.5 +/-8.8 kilojoules/kg/day), in energy intake. Williams' et al. (1999) energy intake findings are suggestive that the shortened luteal phases may be due to an accumulation of moderate energy-deficits or to a specific energy deficit threshold. However, the energy deficit association is confounded by the stress of an abruptly increasing rate in training.

In contrast, training studies that have implemented progressively increasing running programs appear to show no modification of menstrual cycle rhythms. Rogol et al. (1992) randomly assigned seventeen untrained participants, with an average gynecological age of 17.8 +/- 0.9 years, to one of two 1-year progressively increasing training programs: one training program corresponding to a velocity at the lactate threshold (LT) and the other halfway between LT and peak running velocity. Six control subjects, who were recruited separately, were not assigned to any exercise options. Although Rogol et al. (1992) concluded that the 1-year training programs, which increased gradually by 2km every other week with interspersed maintenance weeks, did not adversely affect the participants' robust menstrual cycles; it is of note that the above LT group's luteal phase length did significantly ($p = 0.04$) shorten from an average of 14.4 +/- 0.4 days to 13 +/- 0.8 days. In addition to progressively increasing running programs, Bonen (1992) attempted to randomly assign fifty-seven untrained participants, with an average gynecological age of 17.1 +/- 1.4 years, to one of six experimental running groups: 1) 16km per week over two months; 2) 16 km per week over 4 months; 3) 32 km per week over 2 months; 4) 32 km per week over 4 months; 5) 48 km per week over 2 months; 6) 48 km per week over 4 months. A true randomization of the training programs proved impossible, as numerous women requested assignment to another group; participants were either unable or unwilling to complete the prescribed distances. Statistical analysis with or without the participants changing groups did not reveal any differences. Bonen (1992) concluded that maintaining jogging volumes of 16, 32, or 48 km per week up to 4 months does not alter menstrual cycle or ovulatory rhythms.

Accordingly, gradually increasing or maintaining training volumes appears to have no affect on the menstrual cycle. However, both studies may have overlooked important menstrual cycle outcomes, as Rogol et al.(1992) only obtained blood samples every 4 months and Bonen (1992) collected blood samples every second menstrual cycle.

As can be viewed in Table 2.8, the drastic contrast of Rogol's gradually increasing and Bonen's maintenance programs with Bullen's abrupt increases of 2.4 km per day suggest that a relationship potentially exist between the rate of increases and menstrual cycle irregularities. Although the association with the rate of training program increases seems logical, the confounding variables of energy availability, total volume, intensity, as well as the participants' gynecological ages limit the between-studies comparisons and consequently weaken such a conclusion. Furthermore, the insignificant findings of the non-abruptly increasing training programs remain uncertain, as DeSouza et al. (1998) contrastingly observed disrupted luteal phase and ovulatory functions in participants maintaining an approximately running distance of 32.4 +/-3.5 km over a 3-months period. In addition to completing comparable training volumes as Bonen's maintenance program, DeSouza's et al. (1998) participants also completed an additional 5 hours +/-0.7 hours of other activities per week. Similarly to Bonen's findings, 45% maintained regular ovulatory menstrual cycles (ExOvul); however, 55% of the participants contrastingly demonstrated luteal phase deficiencies (ExLPD) or anovulatory menstrual cycles (ExAnov). As noted in the "energy availability" section, the anovulatory menstrual cycles appear to be attributable to a deficit in energy availability; whereas, the eighty-four percent of the participants who either maintained regularly ovulatory menstrual cycles or experienced disrupted luteal phases present incongruent energy availability findings. As viewed in the training parameter section, the additional training volume of approximately 5 hours per week (DeSouza et al., 1998) is not likely to be the causal factor of the luteal phase inadequacies. In addition to the review of the results of contrasting rates of training program increases, the comparison of Bonen's and DeSouza's similar maintenance training programs with conflicting results demonstrates the current literature's main limitation, namely the neglected documentation of energy availability, as well as, the rate of training program increases. Furthermore, the incongruent findings surrounding DeSouza's et al. (1998) ExLPD group in reference to the ExOvul group, Loucks' energy balanced groups, and Bonen's recreationally active participants demonstrate the current gap in the existing literature, that is identifying the mechanism under a presumably energy balanced state in gynecologically mature and recreationally active women.

In summary of the energy availability and exercise stress literature: 1) Energy

availability below 25kcal/kgLBM/day explicitly demonstrates the role of energy deficit in changed LH pulsatility and ovulatory function; 2) Energy availability above 25kcal/kgLBM/day reveals conflicting results; suggestive of an alternative mechanism; 3) Short-term abruptly increasing training programs do not appear to disrupt the menstrual cycle; 4) Abruptly increasing training programs over a period of 8-weeks appear to disrupt the menstrual cycle; however, they are notably confounded by energy availability; and 5) The menstrual cycle and ovulatory function appear to be unaffected by maintaining or progressively increasing training volumes; however, DeSouza (1998) ExLPD presents conflicting findings. In attempts to curtail the gap of conflicting mechanisms, there has yet to be a study documenting energy availability in gynecologically mature women, while they concurrently complete an abruptly increasing training program over a sufficient period of time. In the current investigation, while monitoring energy availability, recreationally active women between the gynecological ages of 12 and 27 years completed an abruptly, 22.5%, increasing 8-week training program.

2.3 Mechanism: Energy Availability versus Exercise Stress

The menstrual cycle and hormonal rhythms are controlled by a complex neuroendocrine mechanism: the hypothalamic-pituitary-ovarian axis (HPO axis). As described in Barr and Prior (1994), the gonadotropin releasing hormone or GnRH from the hypothalamus stimulates the release of the gonadotropins, follicle stimulating hormone (FSH) and the luteinizing hormone (LH), from the anterior pituitary gland, via neuroendocrine connections. FSH stimulates the growth and maturation of ovarian follicles; whereas, LH stimulates the theca, ovulation and transforms the ruptured follicle into the corpus luteum. The resulting corpus luteum then secretes progesterone and estrogen during the luteal phase. In women with subtle menstrual cycle and ovulatory disturbances, the HPO-axis is down-regulated via an unknown mechanism, resulting in decreased amounts of steroid hormones and gonadotropins. In particular, women experiencing luteal phase deficient menstrual cycles exhibit a suppression of luteal phase progesterone (Beitins et al., 1991; Prior, Vigna, Schechter, et al.1990; Williams et al., 1999; Shangold et al., 1979; Bonen, Belcastro, Ling, & Simpson, 1981; and DeSouza et al., 1998). Moreover, DeSouza's et al. (1998) observational study demonstrated a decrease in early to mid follicular phase (days 2-12) estrone conjugate (E1C) and Loucks & Thuma (2003) short-term severe energy restriction resulted in a significant decrease in the mean 24-h pooled estrogen. In contrast, Loucks' less severe energy restrictions, Loucks, Mortola, Girton, & Yen's (1989) observational study in cyclic athletic women, and Prior, Vigna, Schechter's (1990) recreationally

active runners did not demonstrate any change in E1C levels. The gonadotropin effects include decreased LH pulse frequency and an increased pulse amplitudes (Loucks et al., 1998; Loucks and Verdun, 1998; and Loucks & Thuma, 2003); decreased FSH excretions during days 2 to 5 of the follicular phase (DeSouza et al., 1998); significantly lower follicular stimulating hormone/luteinizing hormone (FSH/LH) ratios (Beitins et al., 1991 and Bonen et al., 1981); a decrease in FSH during the luteal/follicular transition; and a delay in LH surge or an increase in follicular phase length (DeSouza et al., 1998). Anovulatory cycles further display a decrease in LH mean pulse, FSH (Loucks & Thuma, 2003), as well as, the loss of LH surge (Bullen et al., 1985). The unknown mechanism(s) disturbing GnRH and resulting in the hormonal and gonadotropin shifts has yet to be elucidated in prospective studies. To date, research has explored two primary mechanisms: energy availability and exercise stress. Research has also explored a variety of additional mechanisms, including among others body fat, hyperandrogenicity, prolactin, and endogenous opioid peptides (Ferin, 1999; Loucks, Laughlin, Mortola, Girton, Nelson, & Yen, 1992; and Rickenlund, Carlstrom, Bjorn, Brismar, von Schoultz, & Hirschberg, 2003).

2.3a Energy Availability Mechanism:

Physically active women with luteal suppressed menstrual cycles exhibit signs of chronic energy deficiencies; namely, lower levels of leptin, 3,5,3'-triiodothyronine (T_3), insulin, glucose, as well as mildly elevated levels of growth hormone and cortisol compared with sedentary controls (DeSouza, 2003). As Wade, Schneider, & Li (1996) explain, animals encountering suboptimal levels of energy availability are "required to set priorities for the partitioning of calories...animals preserve those activities necessary for survival (e.g., basic cellular functions, locomotion for foraging, thermoregulation); other, less crucial functions (e.g., growth, body fat stores, reproduction) are sacrificed for the time being." (pg. E2). Among other potential factors, the ovulatory function in physically active women may be suppressed in attempts to conserve energy for daily energetic demands, including exercise. Examples of other potential factors include exercise or emotional stress. To date, researchers have demonstrated an association between the hypometabolic state, menstrual cycle disturbances, and the energy deficient hormones of leptin, T_3, and insulin, as well as, another related compound, ghrelin. Although a direct causal relationship does not appear to exist amongst each hormone or substrate, independently; the leptin, T_3, insulin, and ghrelin research has lead to several hypothesises. Namely, Loucks & Redman (2004) postulate a potential mechanism with the availability of

glucose levels to the brain; and Williams (2003) postulates a complex interplay of central factors, as well as, short- and long-term regulators.

Loucks & Redman's (2004) postulation involving glucose availability is based on the similar leptin diurnal and carbohydrate patterns observed in energy deficient and exercising women. Hilton & Loucks' (2000) short-term investigation revealed that low energy availability, not exercise stress, suppressed 24-h mean ($p< 10^{-6}$) and amplitude ($p< 10^{-5}$) of diurnal leptin rhythm by comparing seven sedentary and nine exercising women over a period of 4-days. The random assignment of two energy availability treatments: restricted (10kcal/kgLBM/day) and balanced (45kcal/kgLBM/day), in combination with 30kcal/kgLBM of exercise per day further revealed a noteworthy interactive effect between exercise and energy availability. Specifically, for similar restricted energy availabilities, the sedentary restricted energy group suppressed 24-h mean and amplitude of leptin to a greater extent than the exercising restricted energy group. Correspondingly, Loucks et al. (1998) observed a similar pattern with carbohydrate levels in exercising versus sedentary women, namely exercising women had a higher carbohydrate level due to blood glucose sparing. The similar carbohydrate and leptin findings with respect to LH pulsatility disturbances lead Hilton and Loucks (2000) to postulate that LH pulsatility may depend on carbohydrate availability, not energy intake or exercise stress. Further, Hilton and Loucks (2000) makes reference to the Boden, Chen, Mozzoli, & Ryan's (1996) study that temporarily reversed suppressed leptin levels by infusing 300-400 kcal/day of glucose. This dose is similar to the requirement of the brain in adult humans. In turn, Hilton and Loucks (2000) further hypothesized that "the smaller effects of low energy availability on LH pulsatility in our exercising women may be explained by a greater availability of glucose to the brain." (pg: E48). It is of note that Hilton and Loucks (2000) short-term investigation postulating a mechanism between glucose availability and LH pulsatility has yet to be investigated in a long-term energy availability study in physically active women.

Williams' hypothesis involving the complex interplay of central, short- and long-term regulators is based on the association of leptin, T_3, insulin, and ghrelin with hypometabolic states. As described above, leptin, a protein secreted by adipocytes that is involved in regulating energy homeostasis, was shown to significantly decrease in association with energy-deprived menstrual cycle disturbances (Hilton & Loucks, 2000). Although the low levels of leptin were associated with LH pulsatility disturbances via low energy states, researchers did not demonstrate a direct causal link. Similarly, low levels of 3,5,3'-triiodothyronine (T_3), a regulator of cellular metabolism and energy expenditure, as well as an inadequate indicator of caloric

intake, has also been associated with menstrual cycle and ovulatory disturbances (Williams, Caston-Balderrama et al., 2001; Williams, Helmreich, et al., 2001; Loucks & Heath, 1994; Loucks et al., 1998; Loucks & Verdun, 1998; Loucks & Thuma, 2003; DeSouza, Van Heest, Demers, & Lasley, 2003). Williams, Caston-Balderrama et al. (2001) and Williams, Helmreich, et al. (2001) demonstrated that T_3 levels were associated with changes in reproductive status in cynomolgus monkeys. Specifically, T_3 levels decreased by approximately 20% with the onset of amenorrhea and increased with caloric supplementation and the resumption of the monkey's menstrual cycle. Moreover, Loucks revealed a significant decrease in T_3 in association with LH frequency disturbances; while, DeSouza et al. (1998) observed lower T_3 levels in ExOvul, ExLPD, and ExAnov in comparison to SedOvul. Although an association between low T_3 levels, energy restriction, and reproduction disturbances appears to exist, research has been unable to establish a direct causal relationship. Furthermore, Williams, Helmreich et al. (2001) reports failing to reverse menstrual cycle disturbances by ingesting exogenous T_3. DeSouza et al. (2003) summarizes that T_3 "probably does not directly control the neuroendocrine axis that maintains the hypothalamic-pituitary-gonadal axis"; however, it appears to "play a key role in serving as a metabolic cue to the functionality of the reproductive axis" (pg 344).

The insulin and ghrelin research fails to demonstrate either an association or a causal relationship with subtle menstrual cycle disturbances. Williams, Lancas, & Cameron (1996) investigated the role of insulin, another metabolic hormone implicated in disturbing GnRH, with respect to LH pulsatility by refeeding nine energy-deprived male rhesus monkeys. The post-meal rise in insulin showed no correlation with the increased caloric intake or with stimulation of LH secretion. Further, Williams observed no differences in the food-induced increases in LH pulsatility between the post-meal insulin rise and the post-meal insulin suppression treatments. Williams' et al (1996) study provides strong evidence against a link between insulin and GnRH mechanism. Ghrelin, a peptide produced by the stomach and gastrointestinal tract to regulate food intake, also shows no apparent link with respect to reproductive function. Based on previous research linking ghrelin to the hypothalamus, DeSouza, Leidy, O'Donnell, Lasley, & Willliams (2004) hypothesized that the peptide may play a role in disturbing reproductive function via LH pulsatility. The observational study compared DeSouza et al. (1998) SedOvul, ExOvul, ExLPD, and ExAnov groups, as well as, an additional eight exercising amenorrhea (ExAmen) participants. Ghrelin was shown to be 100% greater in the ExAmen group; however, there were no notable differences between the remaining exercising and sedentary groups. The increase in ghrelin in the amenorrheic groups suggests that the peptide is potentially involved in

the complete suppression of reproductive function. In relation to more subtle menstrual cycle disturbances, ghrelin appears to have no significant impact.

Rather than an independent contribution of any single energy deficit hormones, Williams (2003) hypothesizes that the modulation of reproductive function by energy balance is potentially regulated by a complex interplay of central factors, long-term regulators, such as leptin and insulin, as well as, short-term regulators such as ghrelin. Similarly, Wade and Jones (2003) describe a complex interaction between secondary metabolic cues (long-term and short-term regulators), which respond to prior primary metabolic cue(s), namely short-term (minute by minute or hour by hour) changes in cellular oxidation of metabolic substrates. As presented in Figure 2.5, Wade and Jones' (2003) animal model mechanism proposes that short-term availability of oxidizable metabolic fuels (primary metabolic cues) are detected in the hindbrain most likely by area postrema (AP) – (detectors). The metabolic status is then relayed from the visceral hindbrain to the forebrain likely via neuropeptide Y (NPY), catecholamines (Epinephrine – E and norepinephrine – NE), and other neurotransmitters. Subsequently, afferents from the hindbrain activate the forebrain neurons containing corticotrophin-releasing hormone (CRH) or one of urocortins, which results in estrous behaviour through an unknown mechanism. Wade and Jones (2003) additionally assert that the activation of the hypothalamic-pituitary-adrenal (HPA) axis, the stimulation of CRH, suggests that the changes in reproductive physiology and behaviour may be secondary to a general stress response. Williams (2003) also acknowledges that other mechanisms may play a role, namely the emotional and psychological stress associated with training.

2.3b Exercise Stress Mechanism:

Although human studies have failed to confirm a causal relationship between stress and subtle menstrual cycle disturbances, researchers have proposed a complex interaction between the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis (Ferin, 1999). In animal studies, the activation of HPA and resultant increases of CRH has been linked to the down-regulation of gonadotropin-releasing hormone, GnRH, pulsatile secretions from the hypothalamus. For example, Xiao, Xia-Zhang, & Ferin (2002) investigated the endocrine and menstrual cycle responses to a 12-d stress challenge in eleven adult female rhesus monkeys. The imposed stresses included a short surgical procedure required to install and remove a headcap; the psychological response to a headcap and tethering system; and to the simultaneous move to an unfamiliar environment. The 12-day stress challenge, imposed in the

follicular and luteal phase independently, resulted in a rapid increase in cortisol levels, as well as, a decrease in luteal phase progesterone and integrated LH secretion. The follicular phase stress challenge further resulted in a prolonged follicular phase and a delay in E_2 peak. The cortisol levels decreased during the remaining 11-days; however, they remained significantly higher than baseline levels. The stress challenge appears to provoke menstrual cycle disruptions by means of increased cortisol secretions. However, the findings are potentially confounded by energy availability. Similar to other stress investigations, researchers report body weight, an insensitive indicator of energy availability, and neglect to document energy intake and output. As such, the stress mechanism remains speculative.

In relation to physically active women, investigators stipulate that specific stresses imposed independently or in combination will activate the HPA axis (Loucks and Horvath, 1984 and Prior, 1996a). As can be viewed in Figure 2.6, Petit and Prior (2000) propose a multi-factorial mechanism comprising of four main stressful factors: physical, emotional, nutritional, and over training. The physical parameters include among others weight cycling, the loss of weight, and illness. Emotional is inclusive of any stressful events in life from a first year in university to the loss of a loved one, as well as worry about weight gain (cognitive dietary restraint). The nutrition dimension includes eating disorders in which food intake is limited or there is vomiting or induced diarrhea. Overtraining refers to the physical parameters or as McKenzie (1995) describes, "fatigue that is out of proportion to the training stimulus..." (pg 526). These imposed stresses result in an increase secretion in the corticotrophin releasing hormone (CRH), which potentially disrupts the HPO axis directly or indirectly through the β-endorphin system. Similarly to Willliams' summation of the susceptibility notion, Petit and Prior (2000) further suggest a possible additive effect of reproduction suppression to occur when stresses are imposed on gynecologically immature women. Specifically, Vollmann (1977) revealed that women are more susceptible to anovulatory cycles or shortened luteal phases 10 to 15 years post menarche and again at 30 to 35 years post menarche.

Distinctively, Loucks and Redman (2004) regard the stimulation of the HPA axis "as part of a more complex mechanism that mobilizes stored metabolic fuels when they are available in sufficient amounts and suppresses physiological processes when they are not" (pg. 467). According to Loucks' et al. (1998) energy availability model, "stressors such as surgery, injuries, burns, and infections (which affect energy availability physiologically) and melancholic depression (which impairs energy availability psychologically and behaviourally through reduced appetite, decreased emphasis on feeding, and sustained anorexia) ... affect LH

pulsatility only through their impact on energy availability" (pg 44). The contrasting stress and energy theories are surmised by Loucks and Redman as follows: "One school of thought proposes that menstrual function is disrupted by stress (whatever that may be) and regards energy deficiency as one particular stressor, another school considers that menstrual function is disrupted by low energy availability and regards low energy availability as nothing less than the factor that all stressors have in common – in short, they consider that stress is low energy availability. (pg 468)." Hilton and Loucks (2004) further hypothesis that stress and energy availability are the same concept; in particular, energy availability is the clear and quantifiable measure of stress.

2.3c Other Mechanisms:

A full review of the alternative mechanisms is beyond the scope of this paper; however, potential mechanisms, among others, include hyperandrogenicity, body fat, prolactin, and endogenous opioid peptides (Ferin, 1999; Loucks et al., 1992; and Rickenlund et al., 2003). Rickenlund et al. (2003) proposed that hyperandrogenism is a potential alternative mechanism underlying menstrual cycle disturbances, as eight of twenty-five oligomenorrhea or amenorrhea (OAM) female athletes demonstrated significantly higher androgens (testosterone, free testosterone, and androstenedione). Although the investigators noted that none of the participants had hirsutism or severe acne (symptoms of hyperandrogenism), retrospective ultrasound examinations revealed that three of seven women in the hyperandrogenism OAM group had signs of polycystic ovaries (PCO). As such, the conclusions of the cross-sectional study are biased by the inclusion of women with PCO. Another postulated mechanism is that menstrual cycle function is maintained at a critical threshold of body fat. As summarized in Loucks, Horvath, and Freedson (1984), the postulation that reproductive function is maintained at a critical 22% of body fat has been criticized for its inaccurate assessment of body fat using only height, weight, and age of menarche. The body fat theory is further challenged as numerous studies have demonstrated that menstrual cycle disturbances occur regardless of the amount of body fat. Moreover, there has yet to be a study establishing a causal effect between critical body fatness and menstrual cycle disturbances (Loucks & Horvath, 1984). In addition to hyperandrogenism and body fat, researchers have also failed to demonstrate a causal relationship between menstrual cycle disturbances and the other plausible mechanisms of hyperprolactinemia (Boyden, Pamenter, Grosso, Stanforth, Rotkis, & Wilmore, 1982) and increased amounts of endogenous opioid peptides (McArthur, 1985). This non-exhaustive review demonstrates that

the mechanism(s), if any, of energy availability and stress, independently; the combination of both models; and other postulated mechanisms remains unknown. Future research controlling both stress and energy availability independently is essential in order to unravel the complex, poorly understood menstrual cycle disturbance mechanism.

2.4 Summary: In summary, the existing research reveals a high prevalence of menstrual cycle and ovulatory disturbances in recreationally active women. Researchers assert that a woman's reproductive system is disrupted below an energy availability (EA) threshold of 25kcal/kgLBM/day, independently of exercise stress (Loucks et al. 1998; Loucks & Verdun, 1998; Loucks & Heath, 1994; Loucks & Thuma, 2003; and DeSouza et al., 1998). Alternatively, researchers also suggest that an abruptly increasing training program over an 8-week period disrupts the luteal and ovulatory function (Bullen et al, 1985; Williams et al., 1999). The energy deficit and exercise stress studies documenting significant changes in menstrual cycle and ovulatory disturbances have explored untrained women between the gynecological years of 7.7 to 14.9. Additionally, the studies have only investigated women with normal menstrual and ovulatory cycles (Bullen et al, 1985; Williams et al., 1999; Loucks et al. 1998; Loucks & Verdun, 1998; Loucks & Heath, 1994; and Loucks & Thuma, 2003). It is not known whether EA and/or the stress of an abruptly increasing training program will further disrupt the luteal, ovulatory, and hormone function in gynecological mature and recreationally active women with normally ovulatory and disturbed menstrual cycles. This prospective study will be the first to explore an abruptly increasing training program in gynecologically mature and recreationally active women with normal menstrual cycles and menstrual cycle and ovulatory disturbances. The present study will also be the first to address a segment of the unexplored notion of the rate of exercise program increases while monitoring energy availability.

Table 2.1
Percentages of menstrual cycle and ovulatory disturbances (MCOD) and regular menstrual cycles (RegMC) in recreationally active women (%; SEM)

Study	N	Gynecologic Age (yrs.)	MCOD (%)	RegMC (%)
DeSouza et al., 1998	24	14.9 (1.2)	55	45
Prior, Vigna, Schechter et al., 1990	66	~	80.3	19.7
Bullen et al., 1985 [a]	28	10 (0.4)	75	25
Beitins et al., 1991	18[b]	9.4 (0.65)	55	45
Prior, Cameron, et al., 1982	14[c]	> 5	66	33
Williams et al., 1999	9	8.13 (0.73)	45	55
Averages	-	-	**62.7 %**	**37.1%**

[a]Percentages only given for Weight-Maintenance Group. [b]Percentages based on n = 36 menstrual cycles.
[c]Percentages based on n = 48 menstrual cycles. ~: Not reported.

Table 2.2
Percentages of decreased luteal phase progesterone (↓LPP); shortened luteal phases (SLP); combination of SLP and ↓LPP; and anovulatory (Anov) menstrual cycles in prospective training studies

Study	↓LPP (%)	SLP (%)	SLP + ↓LPP (%)	Anov (%)
DeSouza et al., 1998	8	21	14	12
Prior, Vigna, Schechter et al., 1990	~	60.1	~	19.7
Bullen et al., 1985[a]	~	66	65	42
Beitins et al., 1991[b]	16	39	55	~
Prior, Cameron, et al., 1982[c]	~	33	~	33
Williams et al, 1999	~	45	45	0
Average	**12%**	**44%**	**44.75%**	**21.3%**

[a]Percentages only given for Weight-Maintenance Group. [b]Percentages based on n = 36 menstrual cycles.
[c]Percentages based on n = 48 menstrual cycles. ~: Not reported.

Table 2.3
The average menstrual cycle length (MCL), follicular phase length (FPL), luteal phase length (LPL), and luteal phase index (LPI) in physically active women (Mean; SEM)

Study	N	Gyn. Age[b]	MCL (days)	FPL (days)	LPL (days)	LPI*
DeSouza et al., 1998	24	14.9 (1.2)	26.8 (1.3)	16.35 (0.8)	10.55 (0.4)	0.36[a]
Prior, Vigna, Schechter et al., 1990	66	~	28.2 (2.6)	18.2[a]	10.13 (1.9)	0.36 (0.07)
Rogol et al., 1992	17	17.8 (0.9)	27.2 (1.5)	14.4 (1.2)	12.8 (0.7)	0.48[a]
Bonen, 1992	57	17.1 (1.4)	28.1 (1.3)	15.6[a]	12.6 (1.1)	0.45[a]
Averages	41	**16.6 (1.2)**	**27.6 (1.7)**	**16.1**	**11.52 (1.0)**	**0.41**

[a]approximate calculations based on values given. [b]gynecological age in years.
*Luteal Phase Index (LPI) = luteal phase length divided by cycle length.

Table 2.4
Summary of LH pulsatility effects (%) at various energy availabilities: 10, 20, 30, and 45 kcal/kgLBM/day (Loucks et al, 1998; Loucks & Verdun 1998; and Loucks & Thuma, 2003)

Study/Groups	Codes - see Graph 2.1	EA (kcal/kgLBM/day)	LH pulse Frequency (%)	LH pulse Amplitude (%)
Loucks et al., 1998 Restricted @ 10kcal/kgLBM/d	**R10, 1998**	12.7+/-1.1	↓ 10	↑ 36
Loucks & Verdun, 1998 Restricted @ 10kcal/kgLBM/d	**R10, 1998**	9.9+/-0.2	↓ 57	↑ 94
Loucks & Thuma, 2003 Restricted @ 10kcal/kgLBM/d	**R10, 2003**	9.6+/-0.4	↓39	↑ 109
Loucks & Thuma, 2003 Restricted @ 20kcal/kgLBM/d	**R20, 2003**	19.6+/- 0.1	↓ 16	↑ 21
Loucks et al., 1998 Balanced @ 45kcal/kgLBM/d	**B45, 1998**	46.0 +/-0.8	No significant differences	No significant differences
Loucks & Thuma, 2003 Restricted @ 30kcal/kgLBM/d	**R30, 2003**	29.7+/-0.1	No significant differences	No significant differences
Loucks & Thuma, 2003 Balanced @ 45kcal/kgLBM/d	**B45, 2003**	45.1 +/-0.2	No significant differences	No significant differences

Note. See Table 2.4 for coding and detailed information.
[a]Loucks et al, 1998 study. [b]Loucks & Verdun 1998

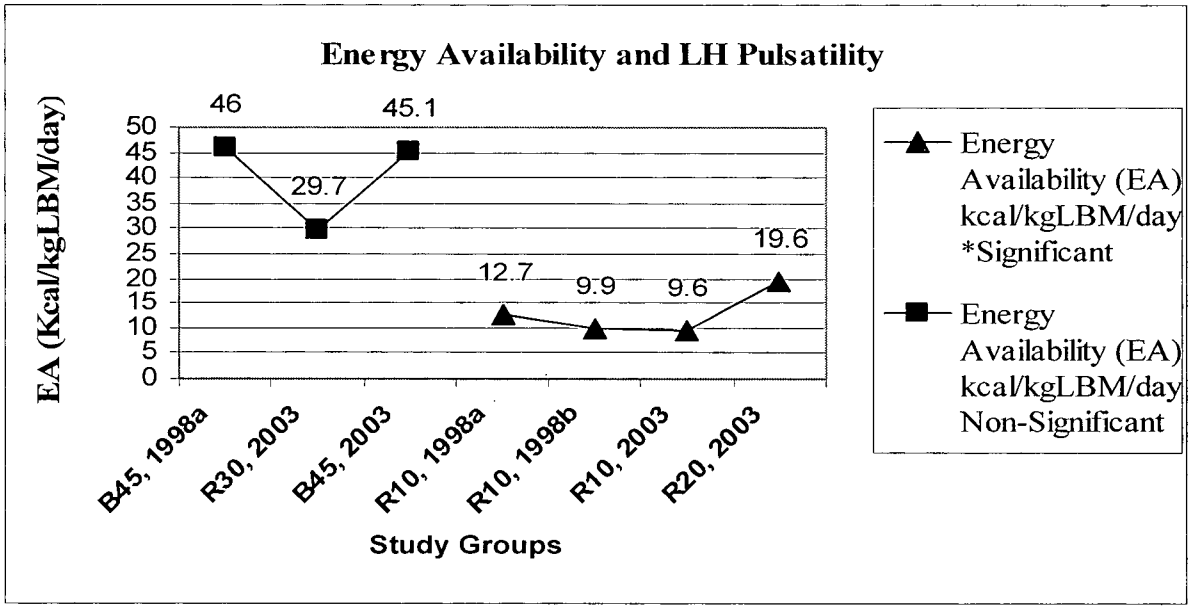


Figure 2.1
Summary of LH pulsatility effects (%) at various energy availabilities: 10, 20, 30, and 45 kcal/kgLBM/day (Loucks et al, 1998; Loucks & Verdun 1998; and Loucks & Thuma, 2003)

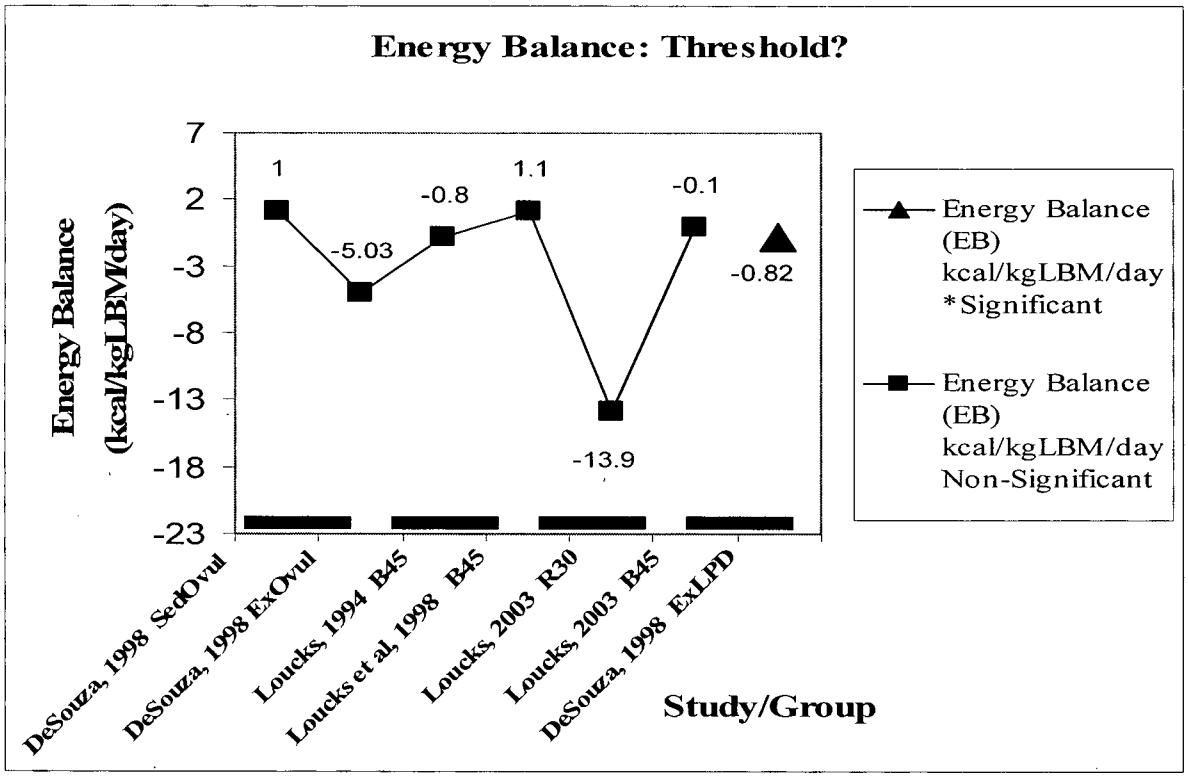


Figure 2.2
Energy balance (EB) comparison between Loucks non-significant groups (1998 and 2003) and DeSouza's groups (1998)

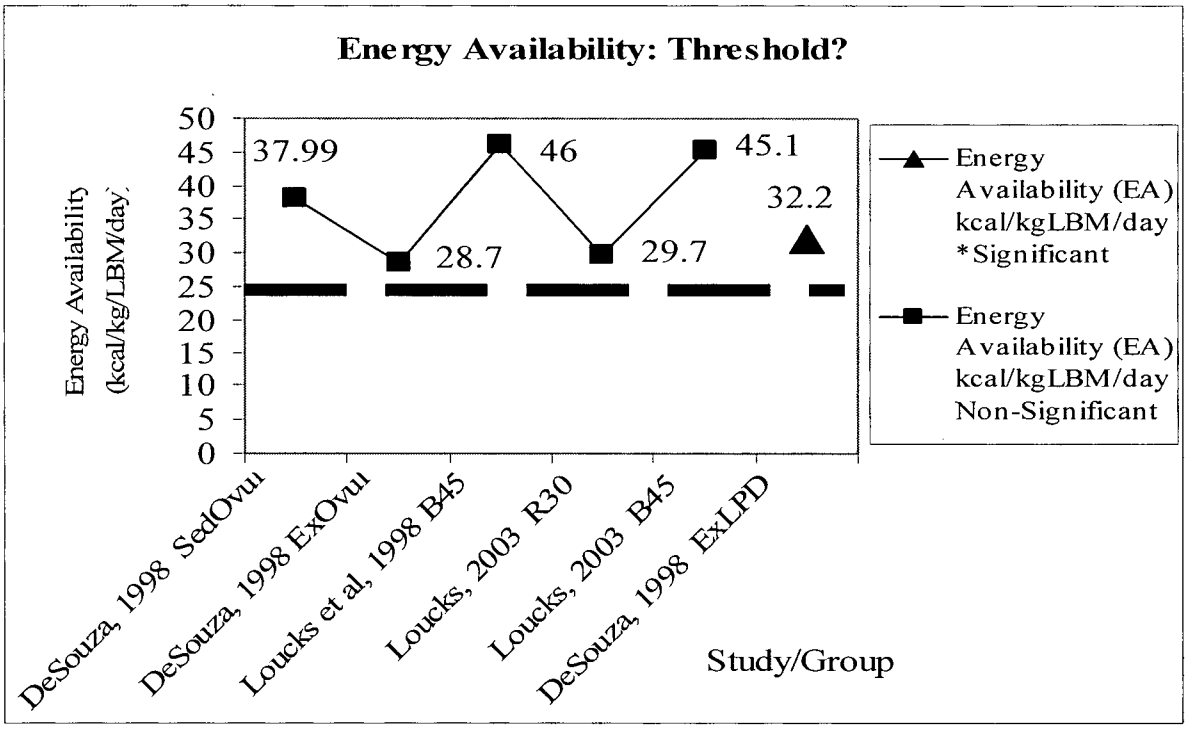
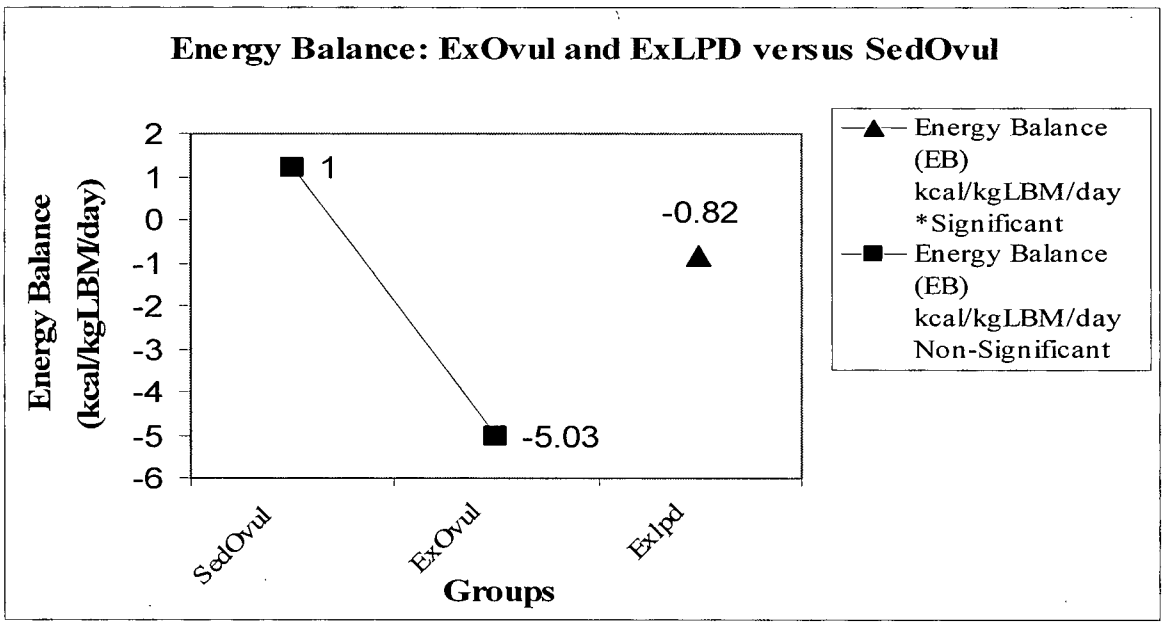


Figure 2.3
Energy availability (EA) comparison between Loucks' non-significant groups (1998 and 2003) and DeSouza's groups (1998)



Note. ExOvul is significantly different than SedOvul; however, ExLPD is not significantly different than SedOvul.

Figure 2.4
Energy balance comparison between SedOvul, ExOvul, and ExLPD in DeSouza et al, 1998

Table 2.5
Ovulatory differences between the combined active group (regular runners and marathon runners) versus normally active group (Prior, Vigna, Schechter et al., 1990)

Ovulatory disturbances	Total (N=66)	Normally Active (N=23)	Active Group (N=43)
Only 1 Short Luteal Phase	12	1	11
More than 1 Short Luteal Phase	28	10	18
Anovulatory cycles	13	3	10

Table 2.6
Additional training parameters in relation to significant or non-significant menstrual cycle disturbances (MCD)

Studies	Intensity	Frequency per week	Duration	Type	Initial Training Status	MCD
Bullen et al., 1985	70 – 80% of Vo2 max	5 (2x/day)	2 months	Run	Untrained	Significant
Bonen, 1992	75 % of Vo2 max[a]	4 – 5	2 – 4 months	Jog	Untrained	Non significant
Rogol et al., 1992	At or above Lactate Threshold	4 - 6	12 – 14 months	Run	Untrained	Non significant
Williams et al., 1999	84 –94 % of max HR (controls – 74 – 91% of max HR)	5	2 cycles	Run or Cycle	Untrained	Significant
Prior, Vigna, Schechter et al., 1990	~	~	1 year	Run – different levels	Varied	Non significant
Prior, HoYuen, et al.,1982a	~	~	12 – 14 months	Rec. marathon runners	Rec. marathon runners	Significant
Prior, Cameron, et al., 1982	~	~	8 months	Rec. marathon runners	Rec. marathon runners	Significant
Shangold et al., 1979	~	~	18 cycles	Chronic running	Chronic runner	Significant

[a]based on regression equation (N=11). ~ not reported.

Table 2.7
Average body weight (kg) changes significantly and non-significantly disturbing menstrual cycles

Length of Study	Weight Change (kg) - Maintained Regular Menstrual Cycle	Weight Change (kg) - Significant Menstrual Cycle Disturbances	Average
< 5 days studies	-0.4	-1.9	**- 1.15**
2-months to 1-year studies	-0.33	-1.45	**- 0.89**
Average	**-0.37**	**-1.68**	

Note. Based on the following studies: Loucks et al., 1998; Loucks & Verdun 1998; and Loucks & Thuma, 2003; DeSouza et al., 1998; Bullen et al., 1985; Beitin et al., 1991; and Rogol et al., 1992.

Table 2.8
The comparison between the rate of training program increases and menstrual cycle irregularities

Study	↑ in Km per day	↑ in Km per week	% Increase range	Outcomes
Rogol et al, 1992	≈ 0.2 km	↑ 2 km every other week	≤ 10 %	Nil
Bonen, 1992	Nil	Nil	Nil	Nil
Bullen et al, 1985	↑ 2.4	↑ 12	↑ 15 – 37.5	Abnormal luteal function and anovulatory cycles
Williams et al, 1999	↑ 2.4[a]	↑ 12 [a]	~	Shortened luteal function

[a] Same rate of training increase as Bullen et al., 1985. ~ Not reported

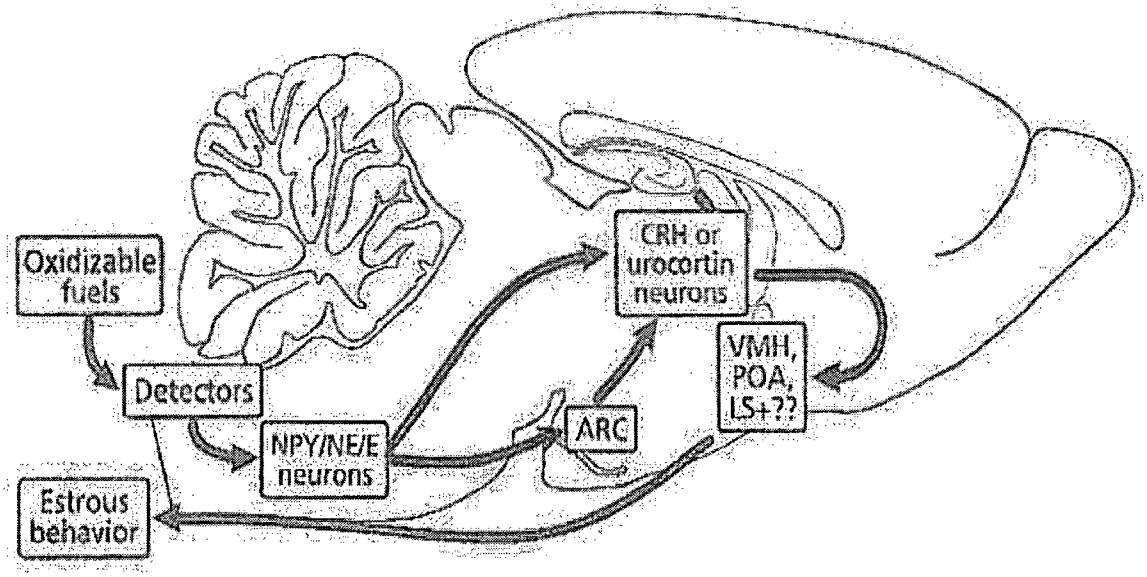


Figure 2.5
Wade and Jones (2003) animal model mechanism: "Working hypothesis as to how decreased metabolic fuel availability inhibits sexual receptivity. CRH, corticotropin-releasing hormone; NPY, neuropeptide Y; E, epinephrine; NE, norepinephrine; VMH, ventromedial hypothalamus; POA, preoptic area: LS, lateral septum; ARC, arcuate nucleus" (pg. 1577).

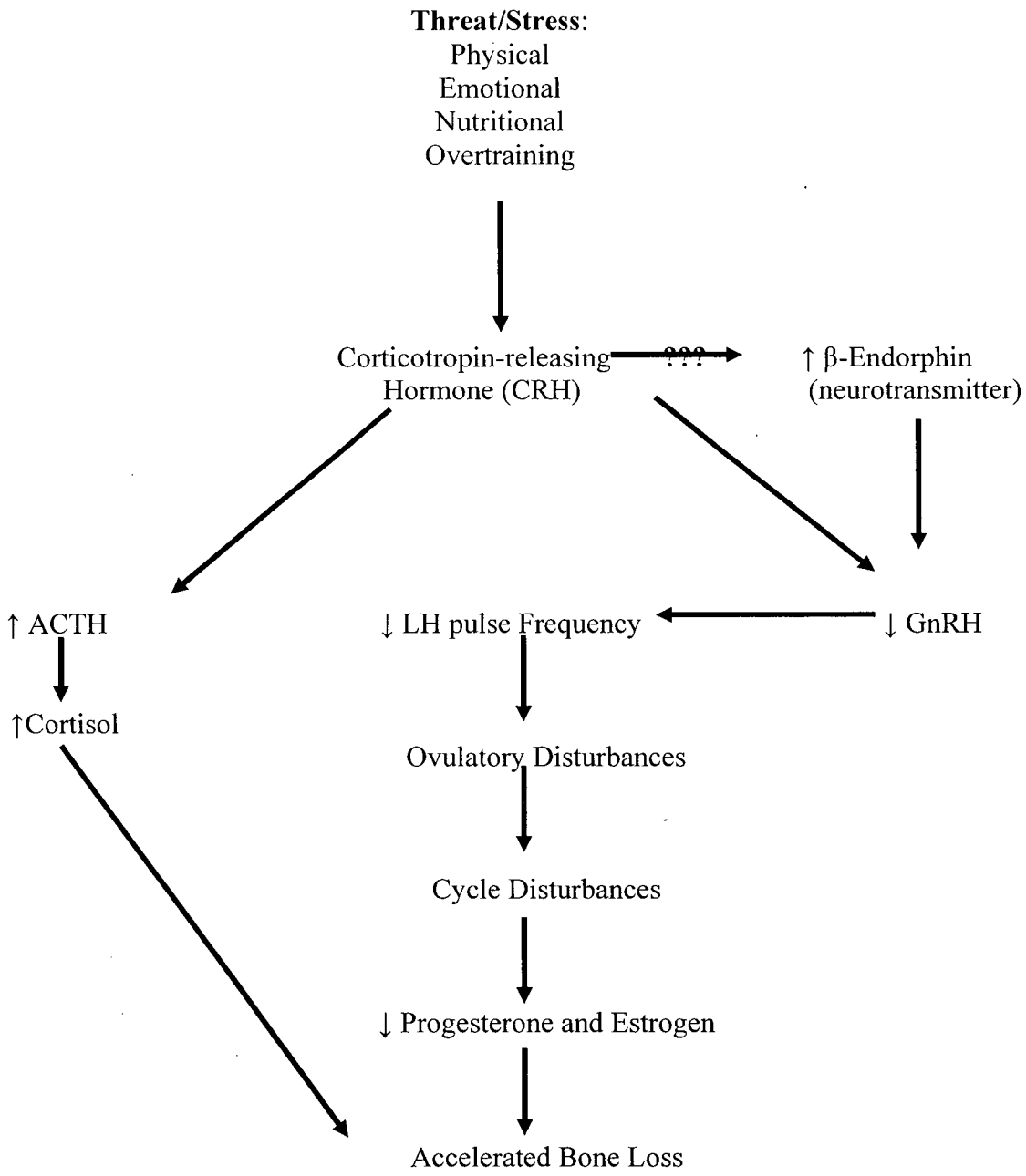


Figure 2.6
Petit and Prior's (2000) multi-factorial mechanism: "Process through which physical, emotional, or nutritional challenges cause increased release of CRH form the hypothalamus. These factors suppress the reproductive system and stimulate the adrenal axis. Abbreviations: ACTH = corticotrophin; LH = luteinizing hormone (Adapted from Prior 36a)" (pg. 141).

Chapter III Methodology

Following ethics approval from the University of British Columbia's (UBC) clinical research ethics board, two hundred and fifty interested women from Vancouver and surrounding areas contacted the investigator in response to newspaper articles, a television interview, flyer postings, or word of mouth. Among these 250 women, 205 were excluded after an initial interview on the basis of the following criteria: gynecological age below 12 or above 27 years; menstrual cycle lengths shorter than 21 or longer than 35 days for the past 3-months; use of hormonal contraceptives in the past 3-months; above recreational activity level (greater than 4 aerobic sessions a week); smokers; attempting to get pregnant; shift workers; low or high body mass index (BMI); a change in weight of more than 2.5 kg during the past 3-months; and restricting caloric intake. Women were also excluded if they had an eating disorder; depression or history of depression; or a cognitive dietary intake score above 13 on the restraint subscale of Stunkard and Messick's (1985) eating behavior questionnaire. The remaining 45 participants attended a group meeting at UBC to sign the information and consent form, complete the questionnaires, and receive instructions on how to use a digital thermometer, polar heart rate monitor, Menstrual Cycle Diary© (MCD), training monitoring forms (TMF), and weekly training logs (WTL). Each participant began the study on the first day of flow of their subsequent menstrual cycle.

Participants maintained daily records of basal body temperatures, physical and emotional symptoms, training sessions, as well as weekly weight over four consecutive menstrual cycles. The four menstrual cycles were divided into the control phase, consisting of the first two menstrual cycles; followed by the training phase, comprising an 8-week abruptly increasing running program. Participants were contacted weekly by e-mail, phone, or in-person throughout the study.

3.1 Participants

The forty-five recreationally active participants were between the gynecological ages of 12 and 27 years and experienced menarche between the ages of 11 and 16 years old. The definition of "recreationally active" included participants who trained between 1-3 times per week at a moderate effort for 30–60 minutes or a vigorous effort for 20-30 minutes (Thomas, Reading, and Shepard, 1992). Eighteen participants completed the 4-menstrual cycle study; two participants completed 3 menstrual cycles; and twenty-five participants dropped out at various times throughout the study.

3.2 Procedures

Figure 3.1 outlines the study's design and illustrates the timing of the urinary samples (U) and dietary records (D) throughout the control and training phases. Morning urinary samples were obtained for pregnanediol-3-glucuronide (PdG) and estrone conjugate (E1C) analysis during each of the following four phases: the early follicular (days 2-5) and luteal phases of the second control menstrual cycles; the luteal phase of the first training menstrual cycle; and the early follicular phase (days 2–5) of the second training menstrual cycle. Cortisol levels were also analyzed from the luteal phase samples. Participants were instructed to collect the luteal phase urinary samples 3-days after the mid-cycle stretchy mucus had appeared and disappeared and/or once the mid-cycle basal body temperatures had increased by 0.3 degrees for 3- to 5-days. If no mucus or temperature patterns were observed, participants were instructed to take the luteal phase urinary samples on menstrual cycle day 21 and every 7 days thereafter until the commencement of the following menses. Participants were also instructed to collect the luteal phase urinary samples when they had not exercised for 24 hours and when they had had an adequate amount of sleep. Each hormone was analyzed independently and between the menstrual cycle phases of luteal and early follicular.

Participants recorded their food and fluid intake for three consecutive days, including one weekend day, within the first ten days of the second control and training menstrual cycles. During the first 7-days of the 3rd menstrual cycle, participants with Physical Activity Readiness-Questionnaire (Par-Q) clearance, blood pressure under 144/94 mmHg, and a resting heart rate under 95 beats per minute, completed baseline maximal aerobic fitness, VO_2 max, and body composition testing in the John M. Buchanan exercise science laboratory. Aerobic fitness and body composition were reassessed either during the follicular phase of the participants' 5th menstrual cycle or the week immediately following the conclusion of the two-month training program. Training monitoring forms (TMF) were completed during every follicular and luteal phase of the control phase, as well as during the follicular phase and every week thereafter during the training phase. Participants were instructed to complete each separate TMF at the same approximate time of day (morning, afternoon, or evening) and the same approximate time of the week (beginning or end). Every participant served as her own control, as each participant's 1st and 2nd training menstrual cycle outcomes were compared to their own control menstrual cycle and ovulatory characteristics.

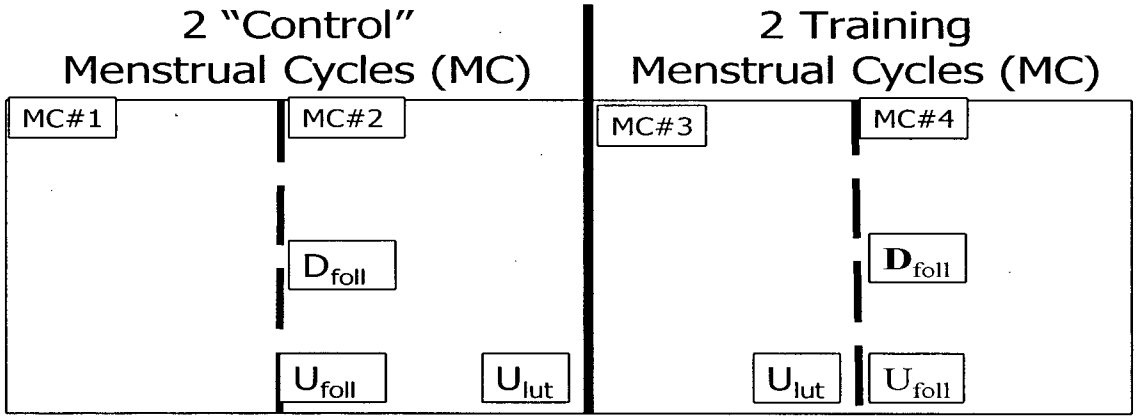


Figure 3.1

Control MC# 1 and 2 versus Training MC # 3 and 4 Study Design. Timing of luteal phase urine sample (Ulut); follicular phase urine sample (Ufoll); and follicular phase 3-day dietary record dietary intakes (Dfoll).

3.3 Training Regimen

The training program began at 20 km per week and increased abruptly in volume and intensity to a final volume of 55.2 km per week. During the first week of the 8-week abruptly increasing training program, the investigator timed each participant around a 400m track. According to each participant's specific running times, the training program in meters, which can be viewed in Appendix C, was then individualized into time (minutes). The example of an individualized training program in Appendix C demonstrates how each participant's regular activities (e.g. soccer practice) were incorporated into the training program. Target running intensities corresponded to the participants' achieved heart rates at ventilatory threshold (VT) during the VO_2 max testing. The high intensity workouts were prescribed above the achieved heart rates at VT; moderate intensity at the achieved heart rates at VT; and low intensity bouts below the achieved heart rates at VT. VT was determined using the excess CO_2 elimination curve method (Volkov et al, 1975). Assessment of ventilatory breakaway was performed using visual inspection by a trained exercise physiologist. Intensity was monitored by Polar heart rate monitors provided by Polar Electro Inc. (Lachine, Quebec) or by heart rate palpitations using the radial or carotid pulse for 10 seconds at the beginning, middle, and end of the training bout.

The total number of km run per week was proportionately divided amongst the three intensities: above VT equaling 28%; @ VT equaling 29%; and below VT equaling 43%. Each component (above, @, and below VT) was increased by 22.5% per week. In terms of volume, participants completed approximately 89.5 km in the first four weeks and 164.5 km during the 5^{th} to 8^{th} week. The abruptly increasing training program consisted of 3 to 7 workouts per week, averaging 6.6 km per exercise bout. Prescribed recovery weeks were interspersed every 4 weeks, as the running program decreased in frequency and duration. Running was the activity of choice, however, cross training was also performed by completing the workouts in reference to the equivalent individualized running times. Cross training included all activities other than running (e.g. cycling and swimming). Participants were instructed to complete 5 to 10 minute warm-up and cool-down sessions, including stretching, at each training bout. The training bouts consisted of continuous, interval, and fartlek workouts. Continuous workouts maintain a constant intensity throughout the session. Interval and fartlek training are similar in that both vary between intensities of above, at, and below VT. However, the duration and intensities of interval running are prescribed, whereas the runner decides upon the duration and intensities of the fartlek intervals.

3.4 Outcome Measures

3.4.1 Menstrual Cycle and Ovulatory Characteristics: Luteal and follicular phase length, ovulation status, and luteal phase index were examined by the quantitative basal temperatures (QBT) method of least-squares analysis (Maximina©; Prior, Vigna, Schulzer, et al.1990). This analysis has been validated in a double-blind study of 24 cycles against the peak serum concentration of luteinizing hormone at midcycle ($r = 0.88$, $p < 0.001$; Prior, Vigna, Schulzer, et al. 1990). Participants' daily temperatures were taken 1 to 3 minutes upon awakening with a digital thermometer (Becton Dickinson). Maximina©; was unable to identify the precise luteal phase length for 6 menstrual cycles (R1 menstrual cycle (MC) #3; R8 MC#1; R31 MC#2; R36 MC#3; R33 MC#3 and #4), as the program chose a luteal onset with a missing temperature data point. The luteal phase lengths of the menstrual cycles with a missing luteal onset data-point were determined by taking the first data-point following the luteal onset day, as determined by Maximina. It is of note that this method may have underestimated the lengths of the luteal phases by one to five days. In terms of categorizing the adjusted luteal phases (regular versus shortened), the luteal phase status did not change in four menstrual cycles. The luteal phase statuses of R1's and R33's 3rd menstrual cycles were classified as shortened (9-days); however, the luteal phase lengths may have been normal (12 to 13 days). Moreover, the ovulation status of R37 MC#2 could not be determined due to missing temperature data points.

The remaining constructs of menstrual cycle length and change in pre-menstrual experiences were evaluated from the participants' Menstrual Cycle Diary© (Prior, 1996a), as can be viewed in Appendix B. The Menstrual Cycle Diary includes the date, basal body temperatures readings, the factors that would affect the temperature readings such as late rising, insomnia, or illnesses, the date of urine sampling, menstrual flow, as well as an evaluation of their daily physical and emotional symptoms. Experiences such as symptoms of breast tenderness, fluid retention, and mood symptoms were assessed on a 0 to 4-point scale. The other symptoms that are never truly absent such as breast size, appetite were evaluated with an alphabetical scale centering around U, for usual and extending to M and L for decreases to Y and Z for increases (Prior, 1996a). The letter scale was transformed into a number scale, ranging from -2 to +2 for statistical analysis. Participants were included in the premenstrual experience analysis if their global pre-menses (7-days prior to the onset of flow) scores were at least 20% higher than their global post-menses (days 8 to 14 post menses) symptom score in the control phase (DeSouza M., Walker, A., Robinson, M., & Bolland, K., 2000). Pre- and post- menstrual global scores were calculated by summing the scores of each of the 4 premenstrual categories in

the control phase: 1) breasts variable (front and side tenderness, and size); 2) moods (anxiousness, frustration, and depression); 3) fluid retention; and 4) appetite. The global pre-menses scores and each of the categories of the participants were then compared between the 1st control menstrual cycle and the 1st training menstrual cycle.

3.4.2 Hormone Analysis: Participants sealed the morning urinary container and labeled it with the participant's code, calendar date, menstrual cycle day, menstrual cycle phase, and comments (view Instructions for Collecting and Storing Urine Samples in Appendix B). The urinary samples were then immediately placed in a home freezer and stored until transported to the lab. At the lab, the samples were thawed and aliquoted into polypropylene minitubes and stored at -80°C until assay (McConnell, O'Connor, Brindle, and Williams et al., 2002). The principal steroids regulating reproduction function in women, E1C and PdG, as well as the primary glucocorticoid produced and secreted by the adrenal cortex, cortisol, were evaluated by competitive enzyme immunoassays (EIAs) technique, as described in O'Connor et al., 2003. The EIAs technique has been validated in university-aged women who were engaged in a variety of different sports (McConnell, O'Connor, Brindle, & Williams 2002). The urinary hormone concentrations were corrected for hydration status and portion of collection by the measurements of urinary creatinine. The creatinine assays were determined by the Jaffe method (Taussky, 1954). Refer to O'Connor et al, 2003 for assay regents and protocol, as well as details regarding sensitivity and inter- and intraassay coefficients of variation for each hormone. Cortisol assay procedures, sensitivity, precision, and cross-reactivities can be viewed in Assay Designs Inc. website (2004).

In the luteal phase E1C and PdG analysis, urine samples were excluded if the sample was taken more than 1-day prior to the QBT luteal onset; and if the sample was taken less than 1-day prior to the onset of the subsequent menstrual cycle. In the cortisol analysis, urine samples taken in the afternoon or evening were excluded. If two luteal phase urine samples were taken by a participant and the timing of both samples was acceptable according to the inclusion criteria, then an average of the two samples was used for analysis.

3.4.3 Activity Level, Training Stress, and Fitness Testing: Participants reported activity levels in the weekly training logs by documenting the date, menstrual cycle day, type of workout, estimated distance run in km, time, heart rates, and any comments for each workout (Appendix B). As described by Banister & Hamilton (1985), training was quantified by calculating the objective measure of training impulse (TRIMP). The stress of the training program was evaluated with the TMFs, which measures an individual's level of overtraining. A state of

overtraining occurs when an individual's fatigue index is out of proportion to the training stimulus, reflecting a failure to adapt to the training stimulus (McKenzie, 1995). The TMF consists of 10 questions, which are divided into four different segments, including a stressor index for medical (MSI), psychological (PSI), training (trSI), and miscellaneous (MiSI) conditions (Appendix B). Each question is scored from zero to four, and the sum of the segments represents a total stressor index (TSI) (McKenzie, 1995). A within-subject comparison of each participant's stressor indexes was performed, as the perception of stress is specific to each individual. The VO_2 max fitness testing protocol consisted of increasing the treadmill's initial speed of 4-miles per hour by 0.5 miles per hour every minute until reaching the maximal testing speed of 8-miles per hour. The treadmill grade was then increased by 2% every minute until volitional exhaustion. The VO_2 max was reported as VO_2 peak, as the majority of the participants were not able to reach their maximum. Other VO_2 peak parameters reported include total time (seconds), VT (ml/kg/min), VT to VO_2 peak ratio (%), and time to VT.

3.4.4 Body Composition, Energy Intake and Availability: Body composition was assessed by the sum of six skinfolds (triceps, biceps, subscapula, suprailiac, anterior thigh, and medial calf), body percent fat, lean body mass, weight, body mass index, and waist to hip girth ratio. Jackson & Pollock's (1985) body density equation using the sum of the tricep, suprailiac, and thigh was used to compute percent body fat. Lean body mass (kg) was calculated as the difference between total weight (kg) and body fat (kg). Energy intake was recorded as accurately as possible in the 3-day dietary forms by documenting the time, date, complete description of food or beverage, and portion size of each consumed food item (Appendix B). The records were analyzed for total caloric intake (kcal) and macronutrients (carbohydrate, proteins, and fat) using the computer program Nutritionist Five Version 1.6 (First Databank, Inc., San Bruno, California, 1998). Participants did not receive any nutritional consulting until the completion of the study. Energy availability was computed as the difference between total caloric intake and exercise energy expenditure. (Loucks & Thuma, 2003). Corresponding with the dates of the 3-day dietary intakes, exercise energy expenditures were calculated by multiplying the duration of exercise by the activity's estimated metabolic equivalent (MET). METs were estimated according to the compendium of physical activities guide (Ainsworth, B.; Haskell, W.; Whitt, M.; Irwin, M.; Swartz, A.; Strath, S.; O'Brien, W.; Bassett, D.; Schmitz, K.; Emplaincourt, P.; Jacobs, D.; Leon, A., 2000). Energy availability was reported in kilocalories per day (kcals/day), kcals per body weight per day (kcals/BW/day), and kcals per kilogram of lean body mass per day (kcals/kgLBM/day).

3.5 Data Analysis

Data was analyzed using Statistical Package for the Social Sciences - SPSS Version 12.0 (SPSS Inc., Chicago IL). The assumption of normality was tested by the skewness, the Shapiro-Wilk test, and outliers in box plots on the baseline menstrual cycle and hormonal scores. Variables that were not normally distributed were transformed, as described in the result's section. Significance was defined with a Bonferroni-corrected alpha level according to the groups of outcome variables (e.g. the group of menstrual cycle parameters included menstrual cycle, luteal phase and follicular phase length, as well as luteal phase index). Outcome measures were analyzed as follows:

Changes in training program parameters (sum of TRIMPS, mean TRIMPS, sum of time, # of workouts, TSI, MSI, PSI, trSI, and MiSI) and weight were analyzed in a 1 (Group) × 3 (Time: 1 – Average of Control menstrual cycles, 2 – training MC #1, and training MC #2) analysis of variance (ANOVA) with a repeated measure on the second variable ($\alpha = 0.05$). Significant main effects of time were analyzed with paired t-tests. Pre- to post- changes in aerobic capacity, ventilatory threshold, and body composition were analyzed by a paired t-test ($\alpha = 0.05$). Dietary intakes and energy availability changes from the 2nd to the 4th menstrual cycles were analyzed with a paired t-tests ($\alpha = 0.05$).

Changes of luteal phase length, menstrual cycle length, luteal phase index, and follicular phase length from the baseline mean of the two "control" menstrual cycles were analyzed in a 1 (Group) × 3 (Time: 1 –Control, 2 – training MC #1, and training MC #2) ANOVA with a repeated measure on the second variable. Significant Mauchly's sphericity tests were analyzed with the Geisser-Greenhouse epsilon. Significance was defined with a Bonferroni-corrected alpha level of $\alpha = 0$ .0125 (0.05/4). Ovulation status was examined using McNemar's chi-square test and significance is defined as $\alpha = 0.05$. A paired t-test analyzed the change in hormones from control to the training phase. Significance was defined with a Bonferroni-corrected alpha level of $\alpha = 0$ .01(0.05/5). Backward stepwise multiple regressions were performed, with change in energy availability (kcal/kgLBM/day), change in body fat percent, change in weight (kg), and change in TRIMPS as the independent variables and the change in early follicular phase E1C, luteal phase length, and luteal phase PdG as the dependent variables. Changes in the premenstrual global score and each premenstrual category score were analyzed by a paired t-test from the control to the training phase ($\alpha = 0.05$).

Chapter IV Results

4.1 Participants: The ability to recruit recreationally active women with regular menstrual cycle lengths (between 21 and 35 days) is presented in Figure 4.1. After the initial screening procedure, eighty-two percent (n = 205) of the two hundred and fifty volunteers were deemed ineligible to enroll in the study. Forty-four percent (n=20) of the remaining forty-five eligible participants completed the study and fifty-six percent (n=25) dropped-out. Overall, eight percent of the 250 volunteers completed the study. The ineligible women and participants who dropped-out will be discussed in section 4.6.

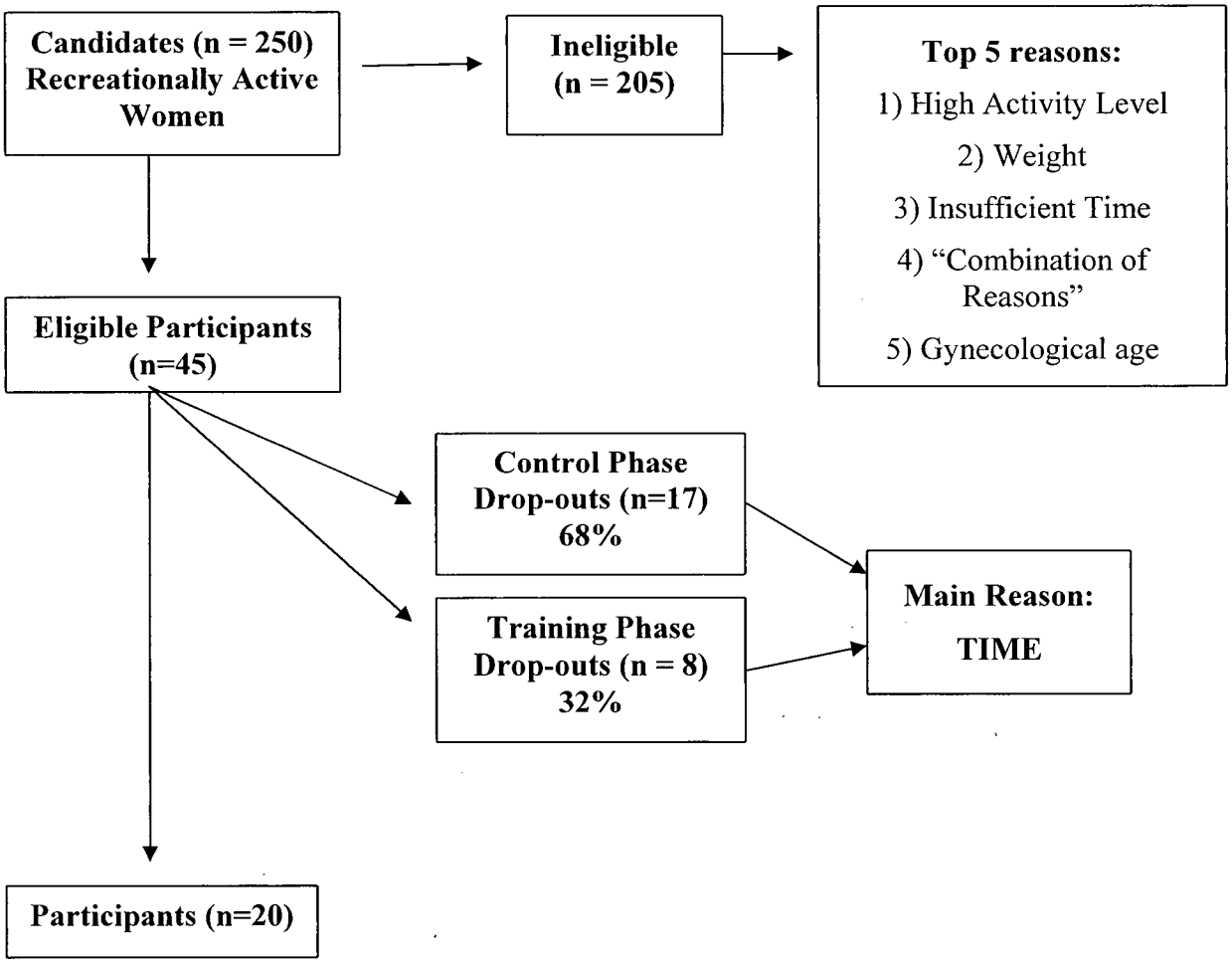


Figure 4.1
Illustration of participant recruitment, ineligible participants, drop-outs, and participants

4.1.2 Demographics: A total of 20 recreationally active women between the gynecological ages of 12 and 27 years completed the 8-week training study. Table 4.1 presents the physical characteristics of the participants: mean age of 32.3 +/- 2.54 years; mean height of 165.5 +/- 2.36 cm; and mean weight of 60.6 +/- 3.96 kg. The cohort comprised mainly of Caucasian women (80%); university graduates (75%); and the majority of the participants were employed with either full (40%) or part time (30%).

Table 4.1
Physical characteristics of recreationally active women (N = 20)

Characteristic	Mean	95% C.I.
Age (yrs)	32.3	(29.7, 34.8)
Gynecological Age (yrs)	19.0	(16.8, 21.2)
Height (cm)	165.5	(163.1, 167.8)
Weight (kg)	60.6	(56.6, 64.6)

4.1.3 Body Composition, Nutritional, and Fitness Characteristics: Nutritionally, participants were not currently restricting calories and 95% had never had an eating disorder (1 participant had bulimia 15 years ago). In terms of activity levels prior to entering the study, participants reported exercising on average 3 times per week. Each workout was between 40 and 75 minutes in duration and completed at low, medium, and high intensities. The mean body composition, nutritional, dietary restraint (CDR), and fitness characteristics during the control phase are listed in Table 4.2.

Table 4.2
Body composition, nutritional, dietary restraint (CDR), and fitness characteristics of recreationally active women (mean +/- 95%C.I.; n = 20)

Characteristic	Mean	95% C.I.
BMI (kg/m^2)	22.1	(20.9, 23.3)
Sum of 6 skinfolds (mm)	107.6	(92.4, 122.7)
Body Fat (%)	22.7	(19.8, 25.6)
Energy (kcal)	1800.3	(1628.1, 1962.6)
CHO (gm)	226.0	(205.1, 246.9)
Protein (gm)	72.2	(60.1, 84.4)
Fat (gm)	66.7	(57.5, 75.9)
CDR	5.7	(4.2, 7.2)
Energy Availability (kcal/kgLBM/day)	36.1	(31.8, 40.4)
Sum of TRIMPS (control phase)	1063.4	(875.5, 1251.3)
VO_2 peak (ml/kg/min); n = 19	37.9	(35.0, 40.8)
VT (ml/kg/min); n = 18	28.6	(26.3, 30.8)

Body composition and fitness variables were measured at the initial fitness testing (follicular phase of 3rd menstrual cycle).

4.1.4 Menstrual Cycle and Hormonal Characteristics: Sixty percent of the participants reported having regular menstrual cycles after menarche, which occurred at the mean age of 13.3 (12.6, 13.0) years old. The participants categorized their current menstrual cycle lengths between 21 to 25 days (15%); 26 to 30 days (55%); and 31 to 36 days (25%). Sixty-five percent were nulliparous and ninety percent had previously taken hormonal birth control methods.

The menstrual cycle characteristics, as measured by the Menstrual Cycle Diary and Quantitative Basal Temperatures, over the two control menstrual cycles (n = 40 menstrual cycles) are illustrated in Figure 4.2 and Table 4.3. Ninety-two and a half percent (n = 37) of the menstrual cycles had a regular menstrual cycle length between 21 and 35 days; 55% (n = 22) of the menstrual cycles were regularly ovulatory (ROMC); 25% (n = 10) had a shortened luteal phase (SLP); and 17.5 % (n = 7) were anovulatory (ANOV). The notable difference between the two control menstrual cycles is the higher prevalence of shortened luteal phases in the first (n = 9) versus the second (n = 1) control menstrual cycle.

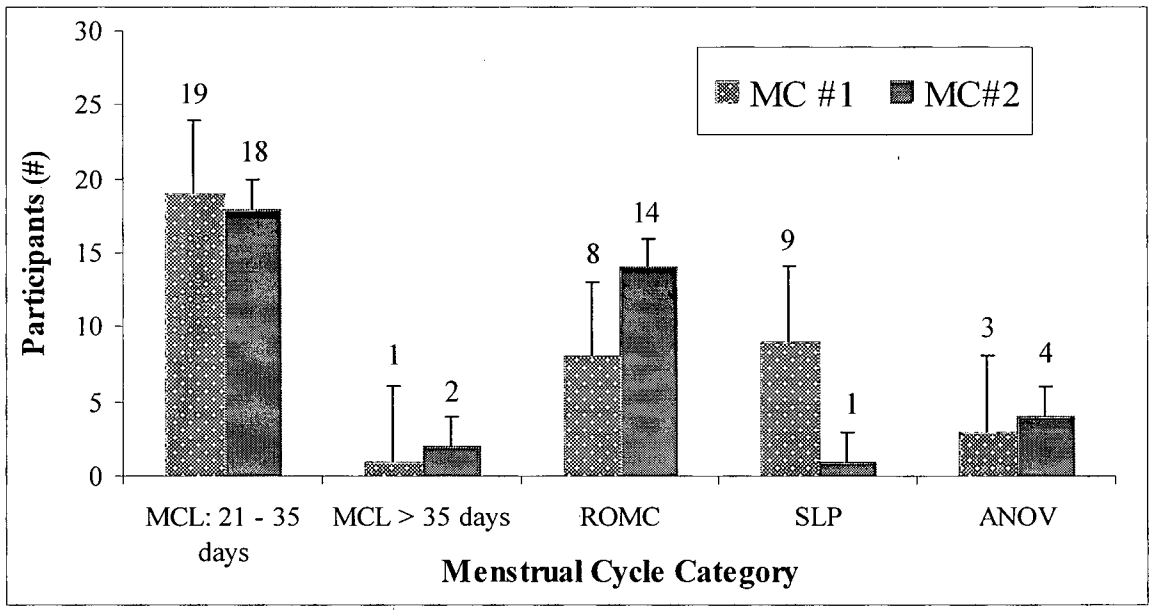


Figure 4.2
The # of participants per MC category during menstrual cycle #1 and #2 of the control phase: MCL: 21 to 35 days; MCL greater than 35 days; regularly ovulatory menstrual cycle (ROMC); short luteal phase (SLP); and anovulatory (ANOV). 95%CI

Table 4.3
Percentage of the menstrual cycle (MC) characteristics in the control phase
(N = 40 menstrual cycles)

MC Characteristics	MC #1 (n=20)	MC #2 (n=20)	Total (n=40)	Percentage (%)
MC Length				
21-35 days	19	18	37	**92.5**
MCL > 35 days	1	2	3	**7.5**
Ovulation Characteristics				
ROMC	8	14	22	**55**
SLP	9	1	10	**25**
Anov	3	4	7	**17.5**

Note: The ovulation status and luteal phase of 1 participant's second menstrual cycle could not be determined.

The ability to recruit recreationally active women with two consecutive regularly ovulatory menstrual cycles is presented in Figure 4.3 and Table 4.4. Menstrual cycle disturbances were prevalent over two consecutive control menstrual cycles, as a mere thirty-five percent (n = 7 participants) maintained regularly ovulatory menstrual cycles. Amongst the 10 participants experiencing at least one menstrual cycle with a subtle menstrual cycle disturbance over the control phase, four participants had 1 ROMC and 1 SLP; one participant had 2 SLP; three participants experienced 1 SLP and 1 ANOV; one participant had 2 ANOV; and one participant had one SLP and one menstrual cycle that could not be determined due to missing temperature data. Three participants had at least one MC with a length greater than 35 days.

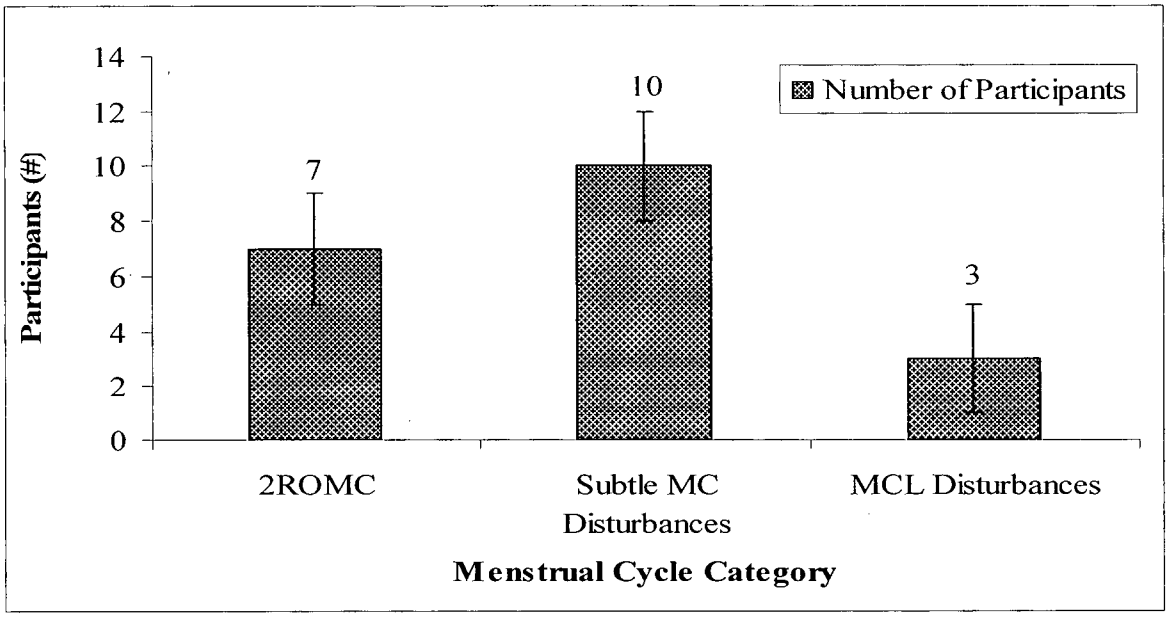


Figure 4.3
The number of participants in each menstrual cycle category: ROMC; subtle MC disturbances; and menstrual cycle length (MCL) disturbances over the two control menstrual cycles. Error bars: 95% CI

Table 4.4
Comparison of participants with regularly ovulatory menstrual cycles and menstrual cycle disturbances over two control menstrual cycles (N=20)

Control MC Characteristics	# of Participants (n)	Percentage (%)
2 ROMC	**7**	**35**
Subtle MC Disturbances	**10**	**50**
1 ROMC & 1 SLP	4	20
2 SLP	1	5
1 SLP&1 Anov	3	15
2 Anov	1	5
1 SLP & 1 Unknown	1	5
MCL Disturbances	**3**	**15**
1 ROMC & 1 ROMC MCL > 35 days	1	5
1 ROMC & 1 ANOV with MCL > 35 days	1	5
1 ANOV & 1 ROMC with MCL > 35 days	1	5

The participants' menstrual cycle and hormonal characteristics during the control phase are described in Table 4.5. Participants presented with a regular mean menstrual cycle (29.5 +/- 2 days) and mean follicular phase (20 +/- 2.6 days) lengths; however, the mean luteal phase was shortened at 8.7 +/-1.7 days. Hormonally, the intra- and inter-assay variability of the urinary analysis was not computed due to the small sample size. The coefficients of variations (CVs) for each hormone are as follows: PdG (7.8% &11.4%); E1C (3.2% & 2.6%); Creatinine (5.1% & 12.3%); and Cortisol (19.3% & 17.4). It is of note that the within-batch cortisol CVs are considerably lower at 1 to 7%.

Table 4.5
Menstrual cycle and hormonal characteristics during the control phase (Mean; 95% C.I.)

MC Characteristics	**Mean**	**95% C.I.**	**n**
Menstrual Cycle Length (days)	29.5	(27.5, 31.5)	20
Follicular Phase Length (days)	20	(17.4, 22.6)	20
Luteal Phase Length (days)	8.7	(7.0, 10.4)	20
Luteal-Phase Index (ratio)	0.30	(0.24, 0.36)	20
Early Follicular PdG (ng/mgCR·)	2856.3	(2136.0, 3576.6)	20
Luteal PdG (ng/mgCR)	12272.1	(9329.2, 15214.9)	16
Early Follicular E1C (pg/mgCR°)	43661.4	(31339.2, 58014.5)	20
Luteal E1C (pg/mgCR)	65353.0	(50549.0, 80157.1)	16
Cortisol (pg/mgCR)	61304.1	(44271.4, 78336.7)	19

· ng/mg CR: nanograms per milliliter creatinine
°pg/mg CR: picograms per milliliter creatinine

Due to a small sample size, women with normal menstrual cycles and menstrual cycle and ovulatory disturbances were analyzed as one group. The differences in menstrual cycle and physical characteristics in the control phase are observed in Table 4.6 between the regularly ovulatory (ROMC) group (n = 7), the shortened luteal phase (SLP) group (n = 6); and the anovulatory (ANOV) group (n = 4). ROMC included participants with two control phase ovulatory menstrual cycles; SLP group included participants with at least one control phase luteal phase less than 10-days; and the ANOV group included participants with at least one control phase anovulatory menstrual cycle. Participants with one shortened luteal phase and one anovulatory phase were included in the ANOV group. Observations in Table 4.6 reveal differences in follicular phase and luteal phase lengths. Notable differences in physical characteristics include the ANOV group's lower mean of gynecological age, total caloric intake (kcals/day), and energy availability (kcals/day), as well as, higher mean physical activity (TRIMPS).

Table 4.6
Exploratory comparison between participants with ROMC, SLP, and ANOV menstrual cycles in the control phase (95% C.I.)

MC Characteristics	**ROMC (n = 7)**	**SLP (n = 6)**	**ANOV (n = 4)**
Menstrual Cycle Length (days)	28.3 (26, 30.6)	27.8 (24, 31.5)	27.6 (23.8, 31.4)
Follicular Phase (days)	16.4 (14, 18.7)	16.8 (10.3, 23.3)	24.75* (22, 27.5)
Luteal Phase Length (days)	11.9 (10.9, 13)	9 (7.7, 10.3)	2.9* (-0.3, 6)
Physical Characteristics			
Gynecological Age (yrs)	19.7 (15.6, 23.9)	20.6 (15, 26)	15 (8.5, 21.5)
Weight (kg)	58.7 (51.5, 66.0)	64.4 (53.9, 75.0)	58.4 (41.5, 75.3)
Body Fat (%)	21.1 (16.3, 25.9)	22.8 (16.6, 29.0)	23.9 (9.7, 38.2)
VO_2 peak (ml/kg/min)	42.3 (38.2, 46.4)	34.9 (30.1, 39.6)	36.4 (23.3, 49.7)
TRIMPS per week	85.7 (47.7, 123.8)	84.3 (33.7, 134.8)	109.4 (37, 181.8)
Total Caloric Intake (kcal/day)	1892.1 (1700, 2085)	1906.5 (1646, 2167)	1664.2 (1381, 1948)
Energy Availability (kcal/day)	1692.2 (1456, 1929)	1759.1 (1445, 2073)	1569 (1276, 1862)
Energy Availability (kcal/kgLBM/day)	37.1 (31.9, 42.2)	36.8 (29.1, 44.6)	36.3 (28.8, 43.9)

*ANOV group: Follicular phase equals menstrual cycle length and luteal phase equals 0

4.2 Training Program Outcomes

4.2.1 Training Program: Changes in training program parameters and weight per menstrual cycle were analyzed in a 1 (Group) × 3 (Time: 1 – Control Phase, 2 – Training Menstrual Cycle (MC) #1, and Training MC #2) ANOVA with a repeated measure on the second variable. Adjustments were made to the sum of TRIMPS and weight ANOVA results using the Geisser-Greenhouse epsilon, as Mauchly's sphericity assumption was violated. Table 4.7 shows the significant increases in training volume and intensity (TRIMPS, time, and number of workouts) from the average of the two control menstrual cycles to the training MC #1 and training MC #2: sum of TRIMPS per menstrual cycle ($F(1, 17) = 12.215$, $p = 0.001$); mean of TRIMPS per menstrual cycle ($F(1, 17) = 7.8$, $p = 0.013$); sum of time per menstrual cycle ($F(1, 17) = 8.932$, $p = 0.008$); and the number of workouts per menstrual cycle ($F(1, 18) = 16.5$, $p = 0.001$). Paired t-test comparison of the main effects of time revealed significant increases between control to training MC #1 and #2. Figure 4.4 illustrates the significant ($* p < 0.05$) changes in training program variables across time. Table 4.7 also reveals that as the volume of training increased, weight in kilograms (kg) did not significantly change over time ($F(1, 15) = 1.056$, $p = 0.321$).

Table 4.7
Change of TRIMPS, time, and weight from control menstrual cycles to training MC #1 and #2 (mean; 95% C.I)

Characteristic	Control MCs	Training MC #1	Training MC # 2	p-value
Activity Level Variables (n = 18)				
Sum TRIMPS	1081.5 (874.1, 1288.8)	1511.7 (1199, 1824)	2157.7 (1620, 2695)	0.001*
Mean TRIMPS	89.3 (67.5, 111)	85.7 (70.9, 100.5)	116.4 (90, 143)	0.013*
Sum of Time	660.5 (642, 679)	772.8 (756, 789.6)	1004.9 (987, 1023)	0.008*
# of workouts (n = 19)	13 (12.9, 13.09)	17.6 (17.5, 17.7)	19.4 (19.3, 19.5)	0.001*
Weight (kg) (n = 16)	64.9 (58.7, 71.1)	61.1 (56.6, 65.6)	61.1 (56.7, 65.5)	0.321

*$p < 0.05$

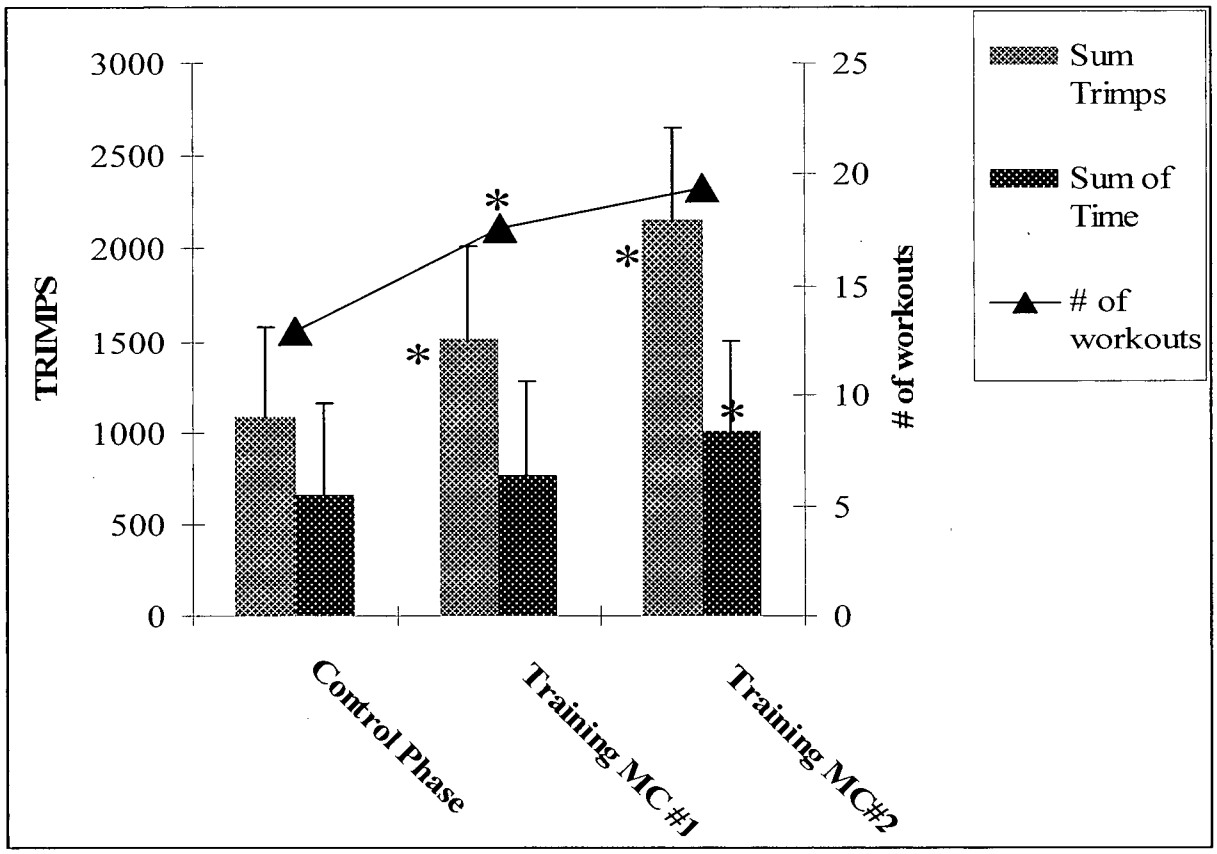


Figure 4.4
Change in Training Program Parameters from the control to training MC #1 and #2. Sum of TRIMPS; Sum of Time (triangles); and # or workouts (squares). C.I. 95%; * $p < 0.05$

4.2.2 Training Stress: Table 4.8 presents the mean changes in the total stressor index (TSI), MSI, PSI, TrSI, MiSI (repeated measures ANOVA). TSI increased insignificantly over time ($F(1, 15) = 1.393$, $p = 0.256$). The medical stressor index (MSI), $F(1, 15) = 6.4$, $p = 0.024$, and the training program stressor index (trSI), $F(1, 15) = 69.544$, $p = 0.00$, significantly increased over time. Paired t-test revealed MSI non-significantly increased from control to training MC# 1 ($p = 0.789$), training menstrual cycle #1 to #2 ($p = 0.144$), and significantly between the control and training MC#2 ($p = 0.017$). TrSI significantly increased from control to training MC#1 ($p = 0.001$), training MC #1 to #2 ($p = 0.036$), and control to training MC #2 ($p = 0.00$). Conversely, the miscellaneous stressor index score (MiSI) significantly decreased, $F(1, 15) = 11.9$, $p = 0.004$. Paired t-test revealed that MiSI decreased from the control phase to training MC #1 ($p = 0.002$) and to training MC #2 ($p = 0.012$), and non-significantly between training MC #1 and #2 ($p = 0.509$). The psychological stressor index score (PSI) did not significantly change over time, $F = 0.747$, $p = 0.449$.

Table 4.8
Mean of stressor index scores over time (mean: 95% C.I.)

Stress Index Variables (n = 16)	Control Phase	Training MC#1	Training MC#2	p - value
TSI	10.6 (7.4, 13.8)	10.3 (7.9, 12.6)	11.7 (9.6, 13.8)	0.256
MSI	3.1 (1.6, 4.5)	3.1 (2.1, 4.1)	4.2 (3.2, 5.2)	0.024*
PSI	4.9 (3.5, 6.3)	4.4 (3.4, 5.4)	4.6 (3.6, 5.6)	0.569
trSI	1.5 (1.1, 1.9)	2.1 (1.6, 2.7)	2.6 (2.1, 3.0)	0.00*
MiSI	1.2 (0.5, 1.9)	0.4 (0.01, 0.8)	0.3 (0.03, 0.7)	0.007*

*$p < 0.05$

Table 4.9 describes the changes over time of the sub-categories for MSI and MiSI stressor indexes. The MSI sub-category describing muscle/joint pain relative to yesterday was the only variable to significantly increase ($p = 0.001$). Muscle/joint pain significantly increased between the control and training MC #2 ($p = 0.001$). The sub-categories of generalized fatigue and sore throat increased insignificantly and change in sleep patterns decreased non-significantly. The MiSI sub-categories of travel zones and irregular diets significantly decreased over time. Travel zones significantly decreased between control and training MC #2 ($p = 0.012$) and irregular diets decreased significantly from the control to training MC #1 ($p = 0.003$) and between control and training MC #2 ($p = 0.011$).

Table 4.9
Means (C.I. 95%) of MSI and MiSI questions over time

Stressor Sub-Categories (n = 16)	Control Phase	Training MC#1	Training MC#2	p - value
MSI				
Generalized Fatigue	0.5 (-0.7, 1.8)	1.2 (0.8, 1.7)	1.3 (1.0, 1.7)	0.251
Muscle/Joint Pain	0.9 (0.5, 1.3)	1.2 (0.8, 1.6)	1.6 (1.1, 2.1)	0.001*
Sore throat, fever, etc.	0.4 (0.1, 0.7)	0.2 (0.03, 0.4)	0.4 (0.1, 0.7)	0.922
Change in Sleep	0.6 (0.2, 1.0)	0.5 (0.1, 1.0)	0.6 (0.1, 1.1)	0.926
MiSI				
Irregular Diet	1.0 (0.4, 1.5)	0.3 (0.04, 0.6)	0.3 (0.02, 0.6)	0.005*
Time zones traveled	0.3 (0.1, 0.5)	0.1 (-0.9, 0.3)	0.0 (0, 0)	0.012*

*$p < 0.05$

Figure 4.5 illustrates the increasing sum of TRIMPS in relation to the increasing total stressor index score (TSI) from the control phase to the 8th week of training (* $p < 0.05$). The increasing mean TRIMPS, rate of increase and percent target increase per week are presented in Table 4.10. Repeated ANOVA revealed a significant increase in TRIMPS over time, $F(1, 14) = 11.347$, $p = 0.00$. Paired t-test between each training weeks revealed a significant increase in training volume from training week 3 to 5 ($p = 0.06$) and a significant decrease in the recovery training week 8 ($p = 0.08$). Training weeks 1, 2, 3, and 6 increased non-significantly and training week 7 decreased in volume of exercise (view Figure 4.5).

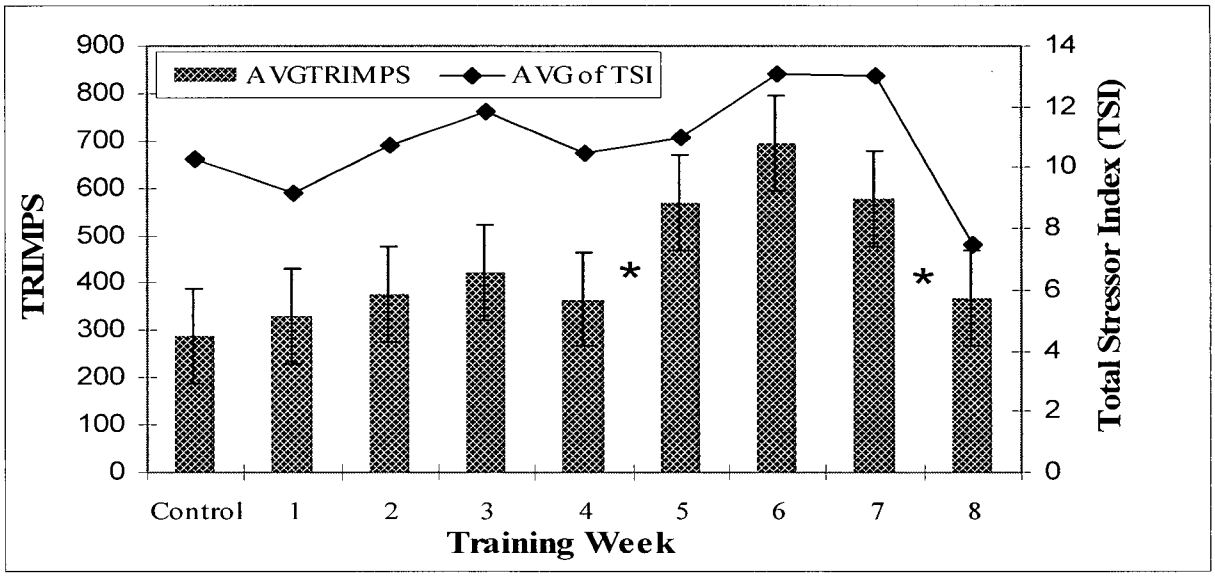


Figure 4.5
TRIMPS versus Total Stressor Index (TSI) score from the mean sum of the control phase to the sum of each training week. Error bars: 95% CI; * $p < 0.05$.

Table 4.10
Mean Sum of TRIMPS from Control Phase to 8th training week (* $p = 0.05$)

Week	Mean TRIMPS	C.I.	p-value	% Increase	Target % Increase
Control	287.6	(231, 344)	-	-	-
1	331.0	(267, 395)	0.246	15.1	22.5
2	378.3	(282, 475)	0.166	14.3	22.5
3	424.2	(324, 525)	0.335	12.1	22.5
4	366.0	(273, 459)	0.303	- 13.7	-
5	570.3	(460, 681)	0.006	34.4[a]	22.5
6	694.2	(513, 876)	0.092	21.7	22.5
7	577.7	(473, 683)	0.175	- 16.8	22.5
8	367.7	(200, 535)	0.008*	- 36.4	-

[a] Percent increase of 3rd to 5th training week.

4.2.3 Fitness Testing: Change in aerobic capacity pre- to post- training program was analyzed by a paired t-test. Table 4.11 displays the mean differences in fitness measures. The 8-week intense training program significantly increased in total time by 27.5 seconds ($t(15) = 2.356$, $p = 0.033$); ventilatory threshold by 2 ml/kg/min ($t(14) = 2.587$, $p = 0.021$); and the amount of time to ventilatory threshold by 33.3 seconds ($t(14) = 2.423$, $p = 0.03$). Although statistically non-significant, $V0_2$ peak improved by 2.3 ml/kg/min ($t(14) = 1.848$, $p = 0.086$). The $VT/V0_2$ ratio did not significantly increase ($t(14) = 0.360$, $p = 0.725$); suggesting that both VT and VO_2 peak similarly increase by amounts of ml/kg/min.

Table 4.11
Change in aerobic capacity pre- post- training program (mean; 95% C.I.)

Fitness Measures	Pre-training program	Post-Training Program	8-week Difference	p-value
Total Time (sec) n = 16	560 (497, 623)	587.5 (528.5, 646.5)	+27.5 (2.6, 52.4)	**0.033***
VO_2 Peak (ml/kg/min) n = 15	37.6 (34.5, 40.6)	39.9 (36.9, 42.9)	+2.3 (-0.4, 5)	**0.086**
VT (ml/kg/min) n = 15	28.7 (26.3, 31.2)	30.7 (28.3, 33.2)	+2.0 (0.3, 3.6)	**0.021***
VT/VO_2 peak ratio (%) n = 15	76.5 (73.6, 79.4)	77.1 (74.7, 79.6)	0.59 (-2.9, 4.1)	**0.725**
Time to VT (sec) N = 15	252 (219.1, 284.9)	285.3 (248, 322.7)	+33.3 (3.8, 62.8)	**0.030***

* $p < 0.05$

4.3 Body Composition, Nutrition, and Energy Availability Outcomes

4.3.1 Body Composition: Table 4.12 reveals the mean body compositional changes pre- to post-training program. The paired t-test shows no significant change in weight, t (17) = -0.412, p = 0.685; body mass index, t (17) = -0.433, p = 0.671; waist to hip ratio, t (17) = -1.058, p = 0.305; sum of 6 skinfolds, t (17) = -0.372, p = 0.714; lean body mass t (17) = 0.539, p = 0.597; and percent body fat t (17) = -0.777, p = 0.448.

Table 4.12
Body composition changes pre- post- training program (Mean; 95% C.I.; n = 18)

Body Composition Variables	Week 1	Week 8	8-week Difference	p-value
Weight (kg)	60.2 (55.8, 64.6)	60.1 (56, 64.3)	-0.14 (-0.83, 0.56)	**0.685**
BMI (kg/m2)	21.9 (20.6, 23.2)	21.8 (20.6, 23.1)	-0.06 (-0.34, 0.22)	**0.671**
Waist/Hip ratio	0.87 (0.85, 0.89)	0.86 (0.84, 0.89)	-0.006 (-0.2, 0.01)	**0.305**
Sum of 6 skinfolds (mm)	104.2 (88.1, 120.3)	103.1 (86.9, 119.3)	-1.07 (-7.1, 5.0)	**0.714**
Lean Body Mass (kg)	46.5 (44.2, 48.8)	46.8 (44.6, 48.9)	0.25 (-0.73, 1.23)	**0.597**
Body Fat (%)	22.1 (19.1, 25.2)	21.5 (18.3, 24.8)	-0.57 (-2.12, 0.98)	**0.448**

$p < 0.05$

4.3.2 Nutrition and Energy Availability: Table 4.13 presents the dietary and energy availability changes from the 2nd to the 4th menstrual cycles. The nutritional variables' paired t-tests did not reveal any significant differences over time: total dietary intake measured in kilocalories (kcal), t (18) = -0.563, p = 0.581; carbohydrate measured in grams (gm), t (18) = -0.951, p = 0.354; protein measured in grams (gm), t (18) = 0.556, p = 0.585; and fat measured in grams (gm), t (18) = -0.93, p = 0.927. The change in energy availability was statistically significant: energy availability (kcal/day) significantly decreased by 205.8 kcals per day, t (18) = -3.168, p = 0.005; energy availability measured in kilocalories per kilogram of body weight (kcal/kgBW/day) significantly decreased by 4.3, t (18) = -3.403, p = 0.003; and energy availability measured in kcal per kg of lean body mass (kcal/kgLBM) decreased by 4.8 (t (19) = -3.249, p = 0.004). Energy availability (kcal/kgLBM/day) remained significant with or without outlier.

Table 4.13
Nutritional changes pre- to post training program (Mean; 95% C.I.; n = 19)

Characteristic	Control Phase	Training Phase	8-week Difference	p-value
Dietary Intake (kcal)	1818.9 (1652, 1986)	1778.9 (1593, 1965)	-40.0 (-189.4, 109.4)	**0.581**
CHO (gm)	229.7 (209.2, 250.3)	218 (191.3, 244.7)	-11.7 (-37.5, 14.1)	**0.354**
Protein (gm)	72.8 (60.1, 85.6)	75.2 (60.7, 89.7)	2.4 (-6.6, 11.3)	**0.585**
Fat (gm)	66.6 (56.8, 76.4)	66.3 (58.2, 74.3)	-0.34 (-8.1, 7.4)	**0.927**
Energy Available (kcal/day)	1662.1 (1488.7, 1835.6)	1456.4 (1270, 1643)	-205.8 (-342.3, -69.3)	**0.005***
Energy Availability (kcal/kgBW/day)	28.9 (24.5, 33.4)	24.6 (20.9, 28.3)	-4.3 (-6.9, -1.6)	**0.003***
Energy Availability (kcal/kgLBM/day)	36.2 (31.6, 40.8)	31.4 (27, 35.8)	-4.8 (-7.9, -1.7)	**0.004***

* $p < 0.05$

4.4 Menstrual Cycle and Hormonal Outcomes

4.4.1 Menstrual Cycle Characteristics: Changes in menstrual cycle parameters scores were analyzed in a 1 (Group) × 3 (Time: 1 – Control Phase, 2 - Training MC #1, and 3 - Training MC #2) ANOVA with a repeated measure on the second variable. Significance was defined with a Bonferroni-corrected alpha level of $\alpha = 0$ .0125 (0.05/4). The menstrual cycle length variable was corrected for normality (log10). As well, the menstrual cycle length was analyzed with and without outliers; there were no statistical differences noted. (Note: In anovulatory menstrual cycles, luteal phase length equals zero and follicular phase length equals the length of the menstrual cycle).

Table 4.14 presents a summary of means for each menstrual cycle parameter across time. The marginal decreases in means from the control phase to training MC #2 were not statistically significant: luteal phase length (LPL), $F(1, 16) = 0.209$, $p = 0.654$, $\eta^2 = 0.013$; menstrual cycle length (MCL), $F(1,17) = 2.802$, $p = 0.112$, $\eta^2 = 0.142$; luteal phase index (LPI), $F(1, 16) = 0.015$, $p = 0.905$, $\eta^2 = 0.001$; and follicular phase length (FP), $F(1 ,16) = 0.052$, $p = 0.822$, $\eta^2 = 0.003$. Practical significance was examined with the strength of association measure, eta squared (η^2). Based on Cohen's criteria of eta squared (0.01 = small, 0.059 = medium, 0.138 = large), the LPL, LPI, and FP main effects of time had a small to medium practical significance and

MCL had a large practical significance. Figure 4.6 shows the graphical representation of the non-statistically significant LPL, FP, and MCL changes.

Table 4.14
Change in mean (95% C.I.) luteal phase length (LPL), menstrual cycle length (MCL), and luteal phase index (LPI), and follicular phase length (FP)

MC Characteristic	Control Phase	Training MC #1	Training MC #2	p-value	Eta squared (η^2)
LPL - days (n = 17)	8.9 (7.3, 10.4)	9.1 (6.9, 11.4)	8.4 (5.7, 11.0)	**0.654**	0.013
MCL - days (n = 18)	29.2 (27.1, 30.9)	28.4 (25.1, 30.5)	27.4 (25.8, 29.0)	**0.112**	0.142
LPI (n = 17)	0.31 (0.25, 0.37)	0.32 (0.24, 0.39)	0.30 (0.21, 0.40)	**0.905**	0.001
FP - days (n = 17)	19.6 (16.6, 22.6)	19.7 (16.8, 22.6)	19.2 (16.0, 22.3)	**0.822**	0.003

$p < 0.0125$

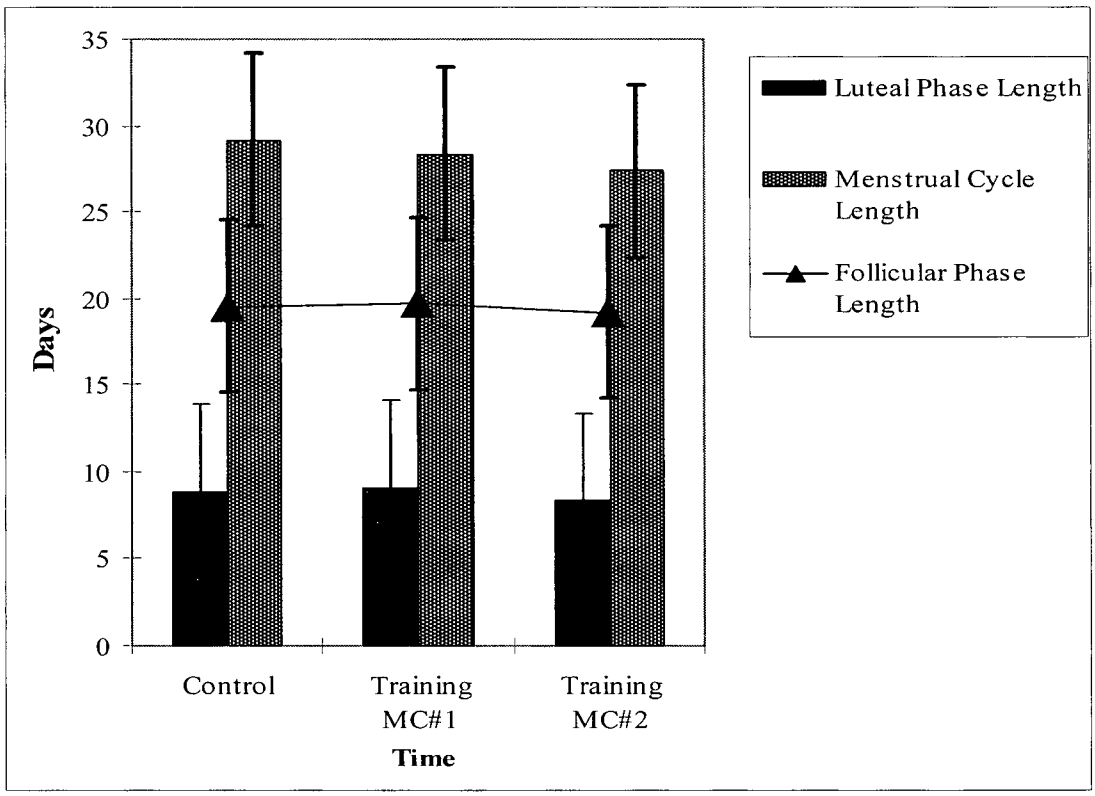


Figure 4.6
Mean changes in luteal phase length, menstrual cycle length, and follicular phase length from control to training MC #1 and #2. Error Bars show 95.0% CI of Mean

4.4.2 Ovulation Status: Ovulation status did not change significantly from the control phase to the training phase menstrual cycles (McNemar's test: p = 1.0; n = 20). The cross-tabulation in Table 4.15 illustrates that 15 participants maintained ovulatory/anovulatory status from the

control to the training phase: 12 participants remained ovulatory; and 3 participants remained anovulatory. Five participants ovulatory/anovulatory status changed from the control to training phase: 2 ovulatory participants in the control phase became anovulatory in the training phase; and 3 anovulatory participants in the control phase became ovulatory in the training phase.

Table 4.15
Ovulation Status; control versus training phase (count)

		Training		Total
		OV	ANOV	
Control	**OV**	12	2	**14**
	ANOV	3	3	**6**
Total		**15**	**5**	**20**

4.4.3 Luteal Phase Length Regression: A backward stepwise multiple regression was performed, with change in energy availability (kcal/kgLBM/day), change in body fat percent, change in weight (kg), and change in TRIMPS as the independent variables and the change in luteal phase length as the dependent variable. The change in luteal phase length was measured from the 2nd control menstrual cycle to the 2nd training menstrual cycle. All predictor models were statistically non-significant. The best predictor model in the backward stepwise regression model was body fat change, b = 0.41, p = 0.167. Figure 4.7 illustrates the luteal phase length regression.

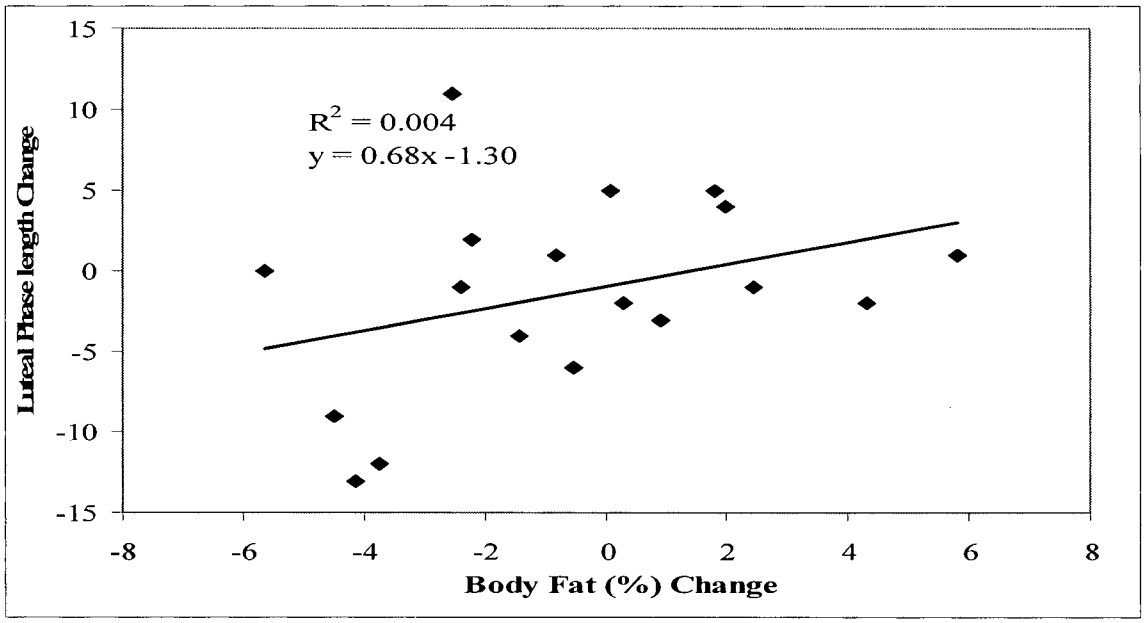


Figure 4.7
Regression - Luteal phase length and percent body fat change

4.4.4 Hormone Characteristics: A Bonferroni-corrected alpha level of p = 0 .01(0.05/5) was used to control for Type I error rate of multiple paired t-tests. Table 4.16 presents the paired t-tests, which revealed no significant differences in early follicular phase PdG, t (17) = 0.639, p = 0.532; luteal phase E1C, t (13) = -0.679, p = 0.509; or cortisol levels, t (16) = 0.500, p = 0.624. Luteal phase PdG displays a trend towards significance, t (13) = -1.942, p = 0.074. Early follicular phase E1C in the training phase was lower than the control phase by a difference of -11288.5 (-20362.7, -2214.3) pg/mgCR, t (17) = -2.625, p = 0.012. The normality of early follicular phase E1C was corrected by taking the square root. Although the mean difference in early follicular phase E1C was not statistically significant with the conservative bonferroni corrected alpha level, it is of note that the p-value, 0.012, is approaching significance.

Table 4.16
Changes in hormones from control to training phase.

Hormones	Control Phase	Training Phase	Difference	P-value
Early Follicular PdG (ng/mgCR) n = 18	2757.3 (1964.4, 3550.2)	3466.9 (1024.6, 5909.2)	+709.6 (-1635.0, 3054.2)	0.532
Luteal PdG (ng/mgCR) n = 14	12920.6 (9708.5, 16132.8)	10446.1 (7048.7, 13843.5)	- 2474.6 (-5227.2, 278.0)	0.074
Early Follicular E1C (pg/mgCR°) n = 18	46119.9 (30922.3, 61306.5)	34831.4 (26007.5, 43655.3)	- 11288.5 (-20362.7, -2214.3)	0.012*
Luteal E1C (pg/mgCR) N = 14	67512.9 (51139, 83886.8)	63010.2 (46577.4, 79443.1)	- 4502.6 (-18837.2, 9832)	0.509
Cortisol (pg/mgCR) N = 17	63634.2 (44744.1, 82524.3)	69216.5 (39740.4, 98692.7)	+ 5582.4 (-18082.9, 29247.6)	0.624

$p < 0.01$
* approaching significance

Figure 4.8 illustrates the change in early follicular phase PdG per participant from the control to training phase. Participant R6 had a large increase in early follicular phase PdG from the 2nd control menstrual cycle to the 4th training menstrual cycle. The participant's menstrual cycles were regularly ovulatory and the urine samples were taken between days 2 and 5 of the early follicular phase. Analysis including R6 resulted in a statistically non-significant mean increase by 709.6 (-1635.0, 3054.2) ng/mgCR, t (17) = 0.639, p = 0.532. Analysis excluding R6 results in a statistically non-significant mean decrease of -348.3 (-1113.8, 417.2) ng/mgCR, t (16) = -0.964, p = 0.349.

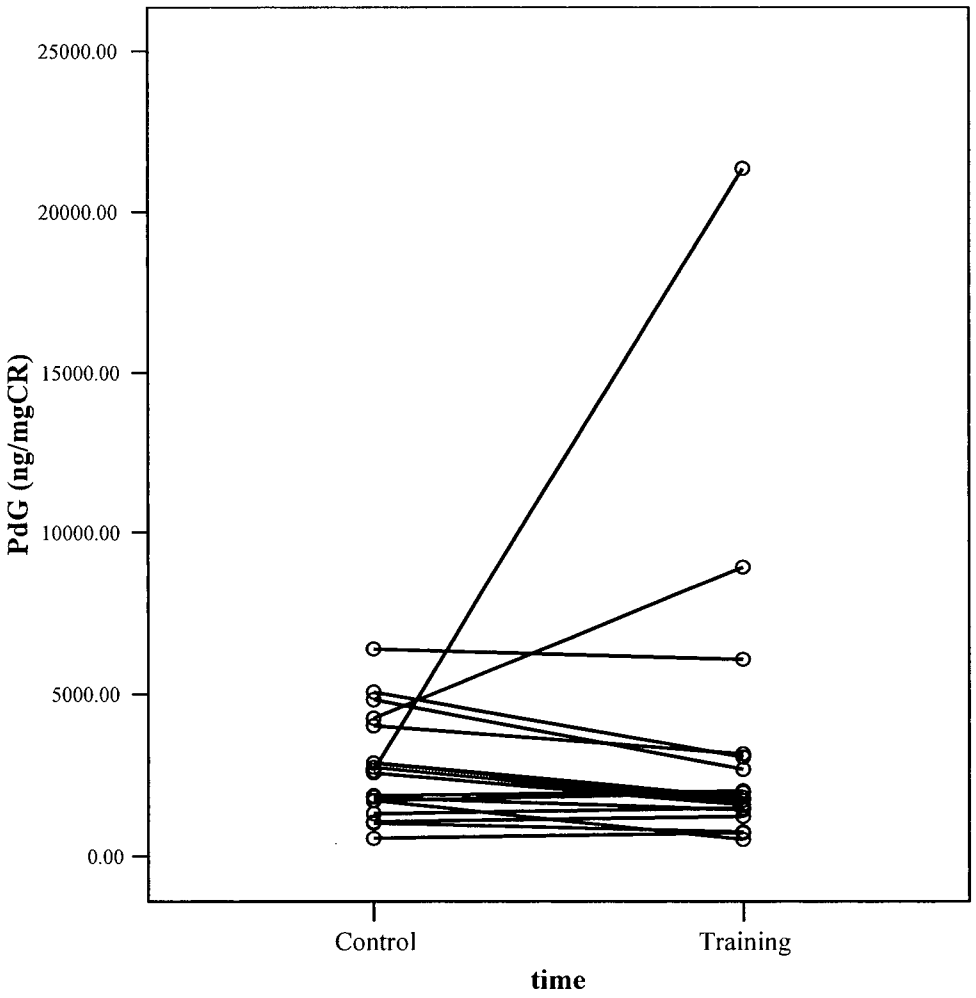


Figure 4.8
Change in Early Follicular Phase PdG (ng/mgCR) per participant from control to training phase

In Figure 4.9, the luteal phase E1C has a varying response with an overall decrease of -4502.6 (-18837.2, 9832) pg/mgCR, p = 0.509. Participant R15 had a large decrease in E1C (pg/mgCR) from 117255 pg/mgCR in the 2nd control menstrual cycle to 41046.2 pg/mgCR in the 1st training menstrual cycle. The participant's 2nd control menstrual cycle was ovulatory with a luteal phase length of 15 days and a long menstrual cycle length of 54 days. The participants 1st training menstrual cycle was ovulatory with a shortened luteal phase of 9 days and a regular menstrual cycle length of 28 days. Paired t-test excluding R15 was also non-significant; however, the mean difference increases by 1013.2 (-7666.2, 9692.7) pg/mgCR, t (12) = 0.254, p = 0.804.

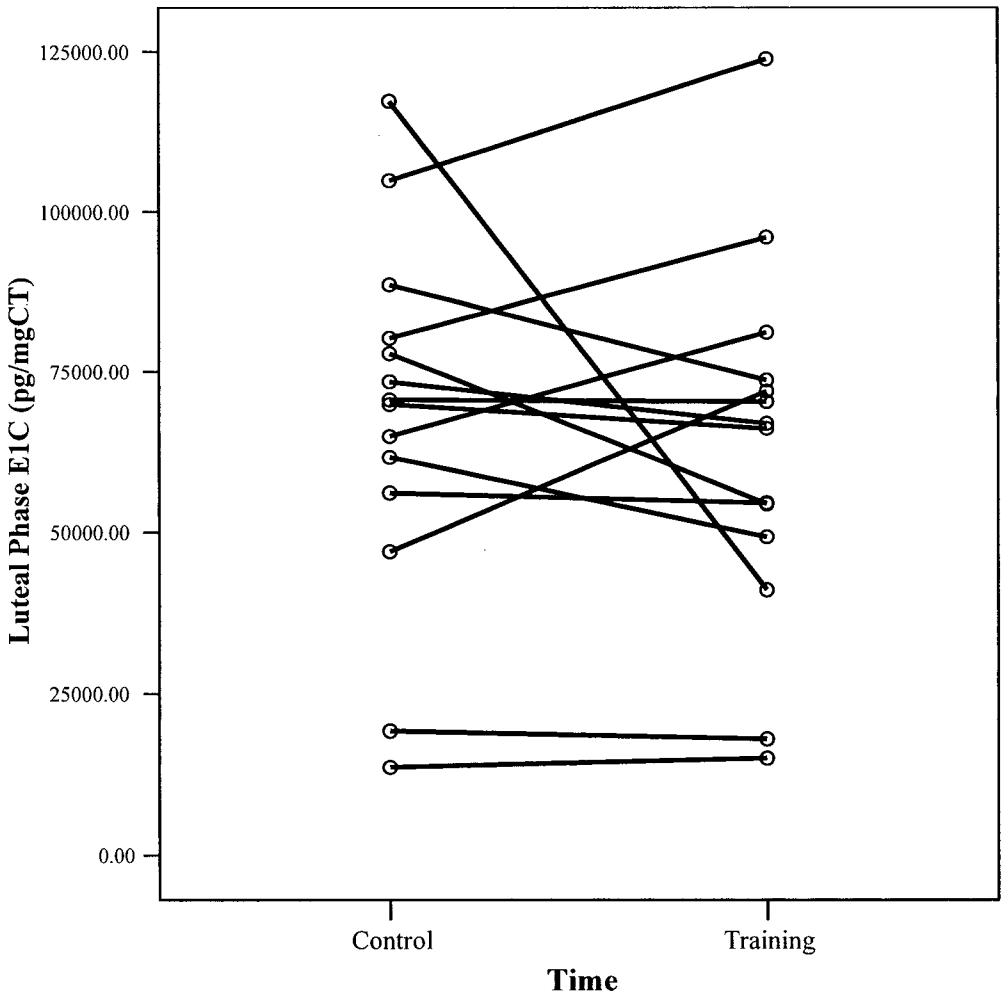


Figure 4.9
Change in luteal phase E1C per participant from the control to training phase

Figure 4.10 illustrates the change of luteal phase PdG per participant from the control to the training phase. Participant R15 experienced a large decrease in luteal phase PdG from an ovulatory menstrual cycle with a long menstrual cycle length of 54 days (control phase) to a shortened luteal phase menstrual cycle of 9-days with a regular menstrual cycle length of 28-days (training phase). Analysis excluding R15 showed a non-significant decrease in mean luteal phase PdG by -1747.4 (-4209.6, 714.9), t (12) = -1.546, p = 0.148.

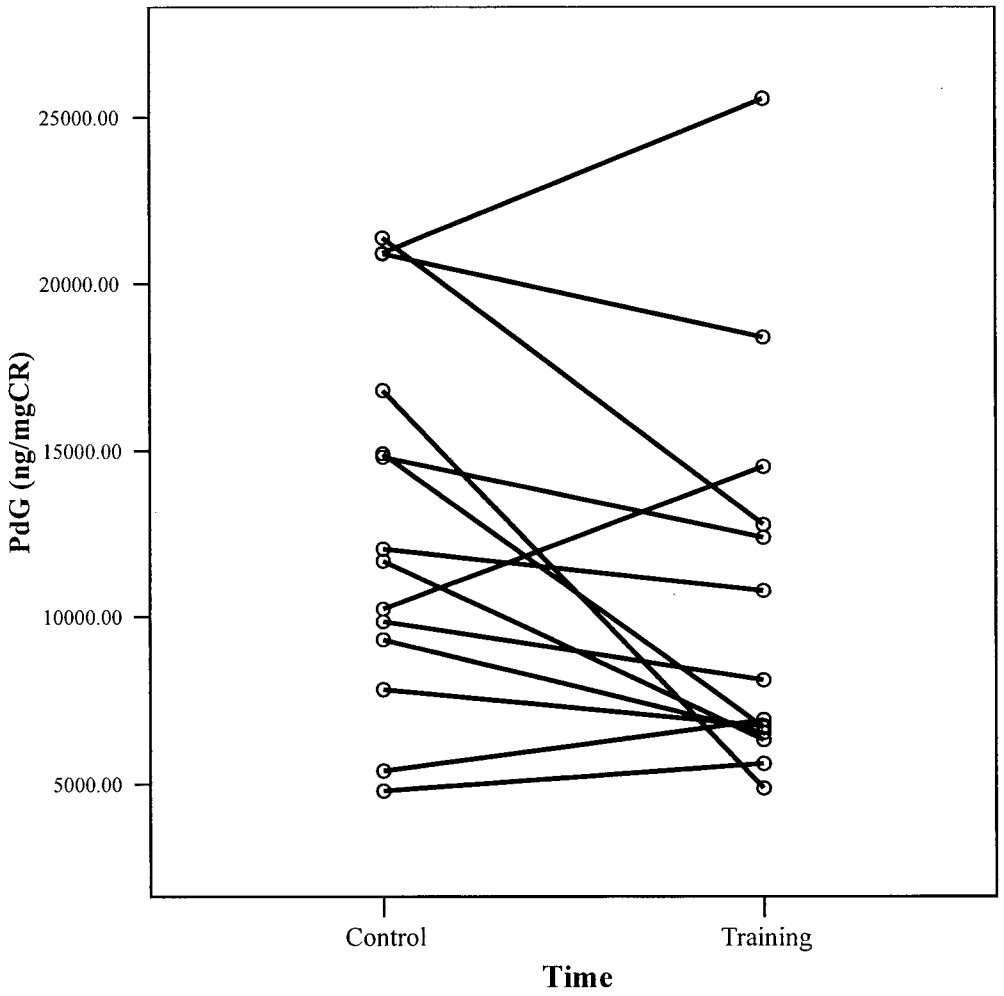


Figure 4.10
Change in Luteal Phase PdG (ng/mgCR) per participant from the control to the training phase

Figure 4.11 depicts the change in early follicular phase E1C per regularly ovulatory (solid lines) and subtle menstrual cycle disturbance (dotted lines) groups. No between groups statistical analysis was performed due to the low sample size. However, the regularly ovulatory group had a greater mean decrease in early follicular phase E1C by -17300.9pg/mgCR as compared to the subtle menstrual cycle disturbances, mean decrease of -4030.71pg/mgCR.

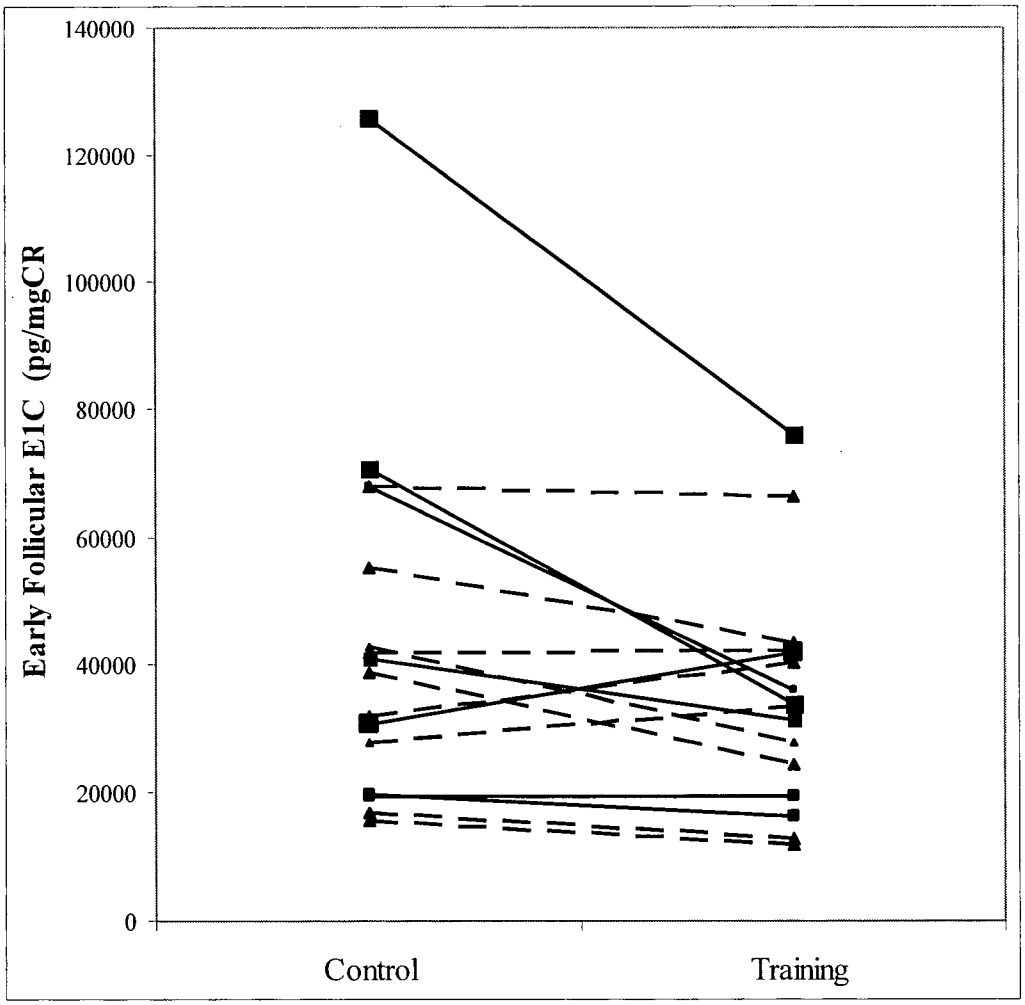


Figure 4.11
Control to training phase change in early follicular phase E1C. Solid lines represent participants with control phase ROMC and dotted lines represent participants with subtle menstrual cycle disturbances in the control phase.

In Figure 4.12, cortisol increases non-significantly by 5582.4 (-18082.9, 29247.6), p = 0.624. Participant R30 has a large increase in cortisol from control phase (61934.6 pg/mgCR) to the training phase (204415.7 pg/mgCR). The participant's menstrual cycle #2 was ROMC and menstrual cycle #3 was ovulatory with a SLP of 9 days. Both urine samples were taken early in the morning between 6 and 7:30am. Analysis without R30 results in a non-significant mean decrease by -2973.8 (-19243.8, 13296.1), t (15) = -0.390, p = 0.702.

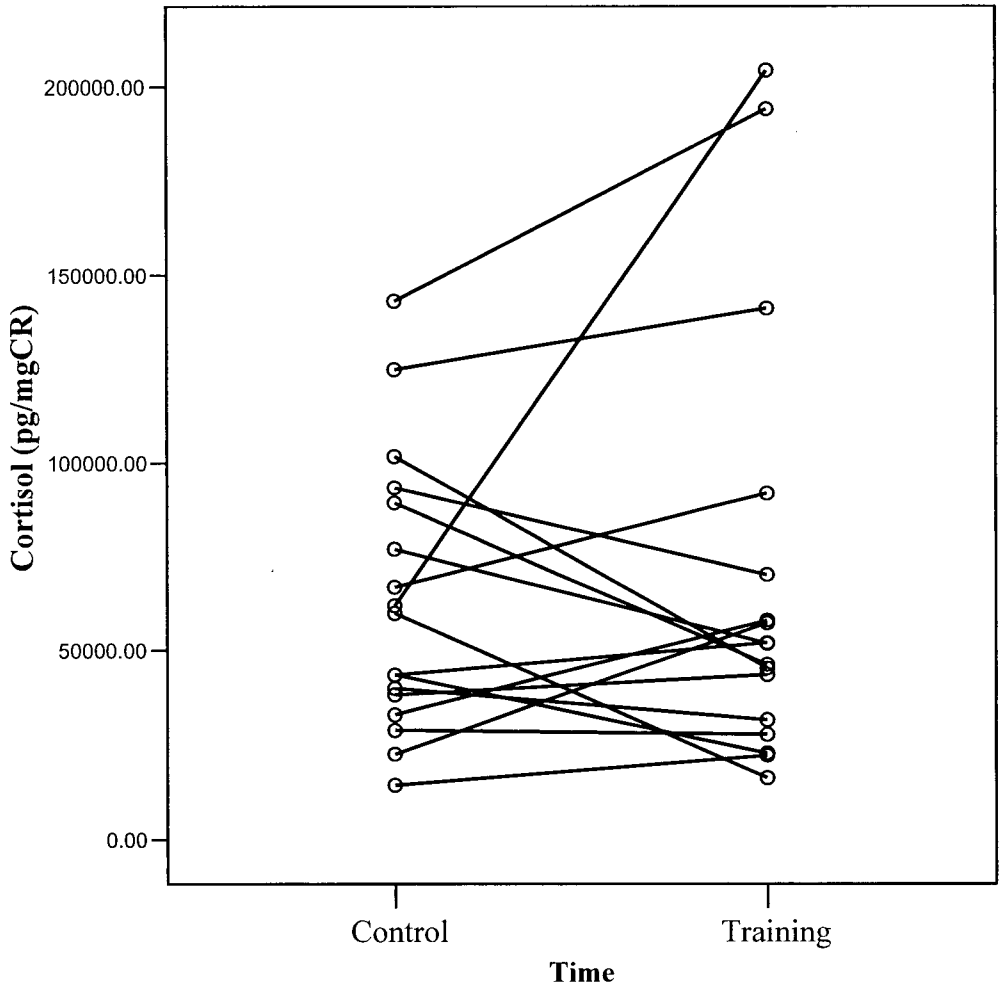


Figure 4.12
Change in Cortisol per participant from the control to training phase

4.4.5 Early Follicular Phase E1C and Luteal Phase PdG Regression: A backward stepwise multiple regression was performed, with change in energy availability (kcal/kgLBM/day), change in body fat percent, change in weight (kg), and change in TRIMPS as the independent variables and the change in early follicular phase E1C as the dependent variable. The change in early follicular phase E1C was measured from the 2nd control menstrual cycle to the 2nd training menstrual cycle. The first predictor variable excluded in the backward stepwise regression analysis was body fat change (b = 0.158, p = 0.829), followed by weight change (b = -0.112, p = 0.665) and TRIMPS change (b = 0.125, p = 0.588). The change in energy availability (kcal/kgLBM/day) was statistically significant (b = -1554, p = 0.044), accounting for 23.1% of the variability in early follicular phase E1C change from the control menstrual cycle #2 to training menstrual cycle #2. Figure 4.13 illustrates the change in early follicular phase E1C (pg/mgCR) from the control to the training menstrual cycles.

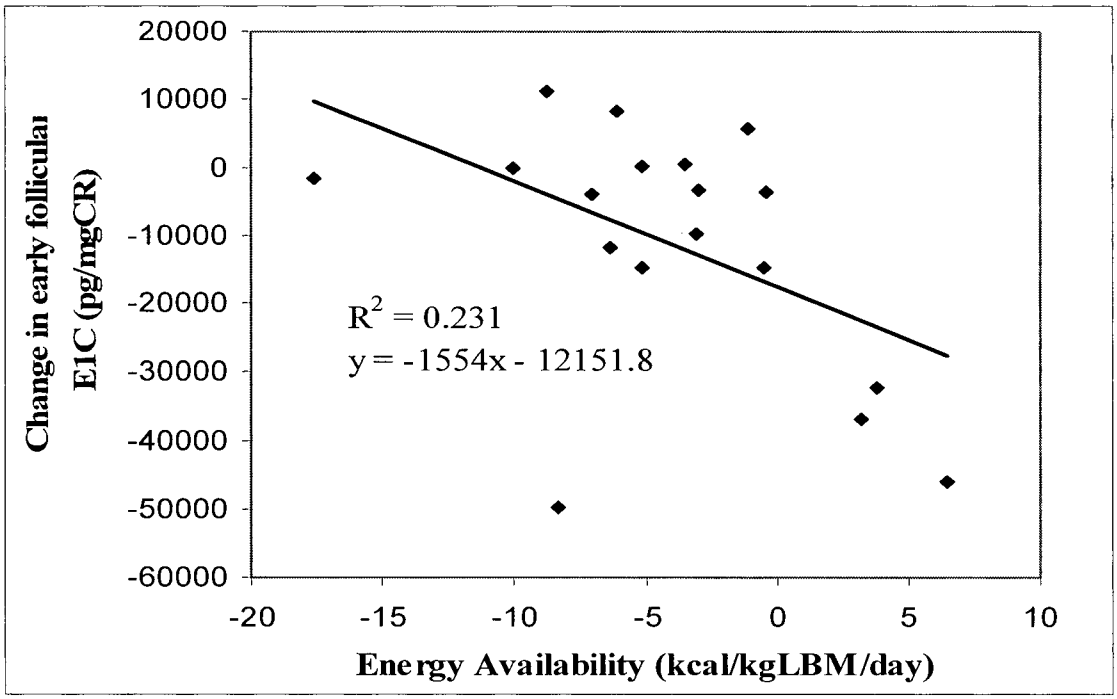


Figure 4.13
Regression - Early follicular phase E1C and Energy Availability Change

A backward stepwise multiple regression was performed, with change in energy availability (kcal/kgLBM/day), change in body fat percent, change in weight (kg), and change in TRIMPS as the independent variables and the change in luteal phase PdG as the dependent variable. Luteal phase PdG did not show any significant regression with change in energy availability (kcal/kgLBM/day), change in body fat percent, change in weight (kg), and change in TRIMPS. The best predictor model in the backward stepwise regression model the change in TRIMPS, b = 2.105, p = 0.083. Figure 4.14 presents the regression scatterplot of luteal phase PdG.

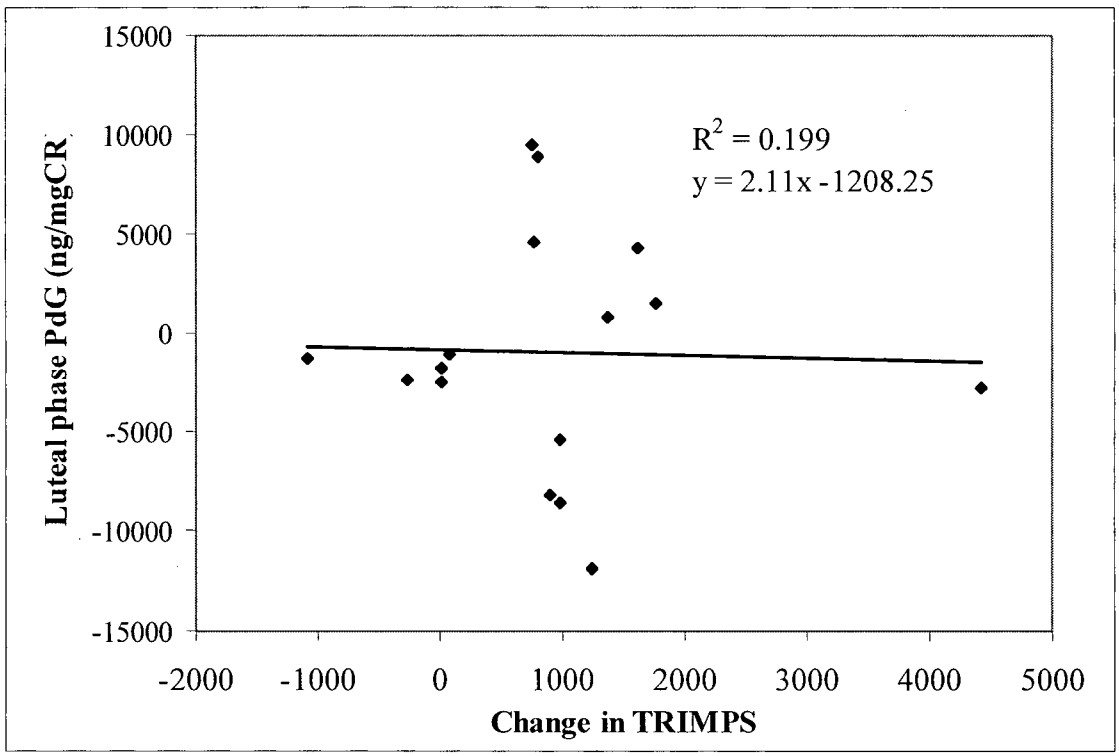


Figure 4.14
Regression - Luteal phase PdG and TRIMPS change

4.5 Pre-Menstrual Symptoms

The change in pre-menstrual symptom global score and each pre-menstrual symptoms category score were analyzed with a paired t-test from the 1st control to the 1st training menstrual cycle (Table 4.17). The global pre-menstrual symptoms mean score did not significantly decrease from the control phase (66.5) to the training phase (56.9), t (12) = 0.729, p = 0.480. Breast score, t (12) = 0.701, p =0.496; energy, t (12) = -1.018, p = 0.329; fluid, t (12) = -0.419, p = 0.683; moods score, t (12) = 0.370, p = 0.716; and appetite, t (12) = -.324, p = 0.752 did not significantly change from control to training.

Table 4.17
Difference scores of pre-menstrual Global and Category symptoms (N = 13)

PMS Symptom	Control to Training Difference	p-value
Breast score: size, front and side soreness	2.7 (-5.7, 11.1)	0.496
Energy	-0.8 (-2.66, 0.96)	0.329
Fluid retention	0.77 (-3.2, 4.8)	0.683
Mood score: anxious, depression, frustration	1.3 (-6.0, 8.6)	0.716
Appetite	0.5 (-2.6, 3.6)	0.752
Global score: breast, moods, fluid, appetite	9.5 (-19.0, 38.1)	0.480

$p < 0.05$

4.6 Ineligible, Drop-outs, and Injuries

4.6.1 Ineligible Candidates: As illustrated in Figure 4.1, two hundred and five candidates were deemed ineligible due to the following five main reasons: 1) high activity levels; 2) weight (changes in weight greater than 5.5 lbs in the past three months, BMI outside eligible ranges, candidates hoping to lose weight during program); 3) insufficient time (candidate was not able to commit to the study due to time or a planned vacation); 4) "more than one reason" (candidates had two or more reasons); and 5) gynecological age (below 12 or above 27 years past menarche). "Other reasons", each equaling less than 3.5%, included currently taking birth control methods; currently or previously experienced a major depression that interfered with their daily life; surgeries; ovarian cysts; wanting group running; family reasons; irregular sleep patterns; worried about injuries or had an injury; pregnant or trying to get pregnant; menstrual cycle lengths greater than 35 days in the past three months; hypothyroidism; and smoking. The remaining 25.9% of the reasons were either undocumented or the reason(s) was/were not provided by the candidates.

4.6.2 Number and Reasons of Drop-outs: As illustrated in Figure 4.1, twenty-five participants (55.6%) dropped-out of the study: 68% discontinued the study in the control phase, prior to starting the training program. The remaining 32% dropped-out during the training phase: five in the 3rd cycle due to sickness (1), hormone intake (1), unknown (1), and time constraints (2); two drop-outs completed less than 75% of the training program; and one participant lost her data. The most prominent reasons for dropping out was time constraints (36%); morning after pill, pregnancy, or back on oral contraceptive pill (20%); injuries before training program (12%); and participants completing less than 75% of the training program (8%).

The demographics and activity level were similar between participants and drop outs. Both groups were comprised mainly of Caucasians (90%) of similar education background (15 participants versus 14 drop-outs had a university degree). Moreover, both groups had similar levels of employment (70% of the participants versus 60% of the drop-outs had part- or full-time jobs). In terms of physical activity, drop-outs exercised, on average, 4 times per week versus 3 times for the participants. The duration of the workouts were similar, ranging from 55 to 60 minutes and both groups completed workouts at varying intensities. It is of note that data was not reported from 7 drop-outs (28%).

4.6.3 Injuries: No participants sustained any injuries throughout the 8-week running program. However, the MSI sub-category describing muscle/joint pain relative to yesterday did significantly increase from control to training phases ($p = 0.001$); refer to Table 4.9 in section 4.2.2.

4.7 Summary of Important Findings

Table 4.18 lists the key statistically significant and non-significant findings. The main menstrual cycle and hormone outcomes that did not significantly change over time include luteal phase length and luteal phase PdG. Levels of cortisol and pre-menstrual symptoms also did not change significantly over time. Early follicular phase E1C approached significance over time and energy availability (kcal/kgLBM/day) significantly accounted for 23.1% of the variability. Concurrently, activity level (sum of TRIMPS) significantly increased; the stress of the training program (TSI) increased non-significantly; body weight remained constant; and energy availability significantly decreased from the control to the training phase. There were no documented injuries throughout the 8-week training program.

Table 4.18
Summary of key statistically significant and non-significant outcomes

Menstrual Cycle and Hormone Outcomes	**p-value**
Luteal Phase Length (days); n = 17	0.654
Luteal Phase PdG (ng/mgCR); n = 14	0.074
Cortisol (pg/mgCR); n = 13	0.705
Pre-Menstrual Syndrome Global Score	0.480
Early Follicular Phase E1C (pg/mgCR); n = 18	0.012
Early Follicular Phase E1C Regression (Energy Availability kcal/kgLBM/day)	23.1% (0.044)*
Training, Body Composition, and Energy Availability Outcomes	
Sum of TRIMPS; n = 18	0.001*
Total Stressor Index (TSI); n = 16	0.256
Weight (kg); n = 16	0.321
Energy Availability (kcal/kgLBM/day); n = 19	0.004*
Injuries (count); n = 20	None

* statistically significant

Chapter V Discussion

This prospective study is the first to explore an abruptly increasing training program in gynecologically mature and recreationally active women with normal menstrual cycles and menstrual cycle and ovulatory disturbances. The present study is also the first study to document the menstrual cycle, ovulatory, and hormonal effects of an 8-week abruptly increasing training program while monitoring energy availability. The results highlight three main findings: 1) Prevalence: supports the current literature reports of an elevated rate of menstrual cycle and ovulatory disturbances, as well as, menstrual cycle status inconsistencies in recreationally active women; 2) Training, Energy Availability, and Menstrual Cycle Parameters: the menstrual cycle parameters, in women maintaining a mean energy availability of 31.4 (27, 35.8) kcal/kgLBM/day, appear to be robust to the stress of an 8-week abruptly increasing training program; 3) Hormones: reveals that the change in energy availability accounted for 23.1% of the variability in early follicular phase E1C decrease from the control menstrual cycle #2 to training menstrual cycle #2.

5.1 Prevalence: This cohort of 20 participants represents women with a mature gynecological age between 12 to 27 years old; healthy body composition with a body mass index between 20.9 and 23.3 kg/m^2 and percent body fat between 19.8 and 25.6%; low cognitive dietary restraint with a mean score of 5.7 on the eating behavior questionnaire; a mean energy availability of 36.1 kcal/kgLBM/day; and a recreationally active fitness level (VO_2 peak of 37.9 ml/kg/min). Amongst this cohort, there is a high prevalence of regular menstrual cycle lengths; a low occurrence of regular ovulatory menstrual cycles, and an elevated prevalence of shortened luteal phases and anovulatory cycles. Furthermore, the physically active women demonstrate a high frequency of inconsistent menstrual cycle status. These control phase observations are congruent with the previous research findings (DeSouza et al., 1998; and Prior, HoYuen, et al., 1982). The contributing factor(s) to the high prevalence of menstrual cycle disturbances remains unclear.

Although relatively robust to change, a low prevalence of menstrual cycle length disturbances does occur in recreationally active women. Over the two control menstrual cycles, 15% (n = 3) of the participants experienced at least one menstrual cycle length greater than 35 days. The mean menstrual cycle length in the cohort was 29.5 +/-2 days; which is consistent with investigators mean length reports of 27.6 +/-1.7 days in recreationally active women (Table 2.3). The prevalence of menstrual cycle length disturbances is also consistent with earlier research: DeSouza et al. (1998) reported excluding 2 of 46 candidates for menstrual cycle

lengths greater than 36 days; and Prior, Vigna, Schechter, et al. (1990) documented 13 of 66 participants experiencing at least one menstrual cycle length greater than 35 days over a 1-year observation period. Prior to entering the study, the 13 participants had previously been screened for two menstrual cycle and luteal phases of normal lengths (Prior, Vigna, Schechter, et al., 1990).

Researchers have yet to establish the causal factor(s) to the lengthening of the menstrual cycle. However, numerous investigators report a high prevalence of menstrual cycle length disturbances amongst athletes competing in leanness sports; including endurance, aesthetic, weight-class, and anti-gravitation sports (Torstveit & Sundgot-Borgen, 2005 and DeSouza, et al.1998). Although speculative and inconclusive, the three participants in this study with menstrual cycle length disturbances were consuming fewer total calories per day (-288 kcals/day) and had a lower energy availability (- 4.7 kcals/kgLBM/day), while completing a similar volumes of activity (+/- 5.8 TRIMPS) than the participants with regular menstrual cycle lengths (n = 17). There were no major differences noted between other possible contributing factors such as gynecological maturity, cognitive dietary restraint, and body fat percentage. It is of note that statistical differences were not computed due to the low sample size (n = 3) and the size difference between groups (n = 3 versus n =17).

In terms of regularly ovulatory menstrual cycles (ROMC) and subtle ovulatory disturbances, a mere seven participants (35%) had two ROMC versus 10 participants (50%) experiencing at least one subtle menstrual cycle disturbance over the control phase. Similarly, the reviewed prospective studies document an average of 37.1% of active women have a regular ovulatory menstrual cycle; whereas, an average of 62.7% will experience subtle menstrual cycle disturbances (Table 2.1). The subtle menstrual cycle disturbances in the reviewed studies included inadequate luteal phase progesterone production, in addition to short luteal phases (SLP) and anovulatory menstrual cycles (ANOV). It is of further note that a greater percentage of subtle menstrual cycle disturbances occurred in the 1st (60%) in comparison to the 2nd (26%) control menstrual cycle (Figure 4.2). In particular, the 1st control menstrual cycle consisted of 8 regularly ovulatory participants, 9 participants with short luteal phases, 3 anovulatory participants. The 2nd control menstrual cycle consisted of 14 regularly ovulatory participants, 1 participant with a shortened luteal phase, and 4 anovulatory participants. Although the explanation for the discrepancy remains illusive, the high prevalence of menstrual cycle disturbances in the 1st control cycle may be a result of an increased "stress" of joining the study or the participants may have altered their lifestyle habits upon entering the study. For example,

the participants may have increased their activity level in preparation for the training program; or decreased their caloric intake in attempts to lose weight. These control phase results demonstrate the importance of documenting ovulatory and luteal function over at least two menstrual cycles before introducing an intervention. More research in needed to confirm the higher prevalence of menstrual cycle and ovulatory disturbances in joining a study and to determine the etiological factors.

The discrepancy between the first two control menstrual cycles is also reflective of the menstrual cycle inconsistencies in recreationally active women. In particular, only 45% of the participants maintained their menstrual cycle status over the two control menstrual cycles: 7 participants had two ROMC; 1 had 2 SLP; 1 had 2 ANOV. In the four observed menstrual cycles, regardless of the 8-week training program, only 6 participants maintained a consistent menstrual cycle status: 5 of the 7 participants with two ROMC in the control cycle; and 1 participant with ANOV control cycles. Congruently, investigators have demonstrated that physically active women display a more inconsistent menstrual cycle status as compared to sedentary controls (DeSouza et al., 1998; and Prior, HoYuen, et al., 1982). In particular, DeSouza et al. (1998) demonstrated that 91% of the sedentary controls had consistent ovulatory cycles over a period of three months; whereas only 54% of exercising women maintained either a consistent luteal phase deficient or anovulatory menstrual cycles.

The latest research asserts that a woman's reproductive system depends on energy availability; that is the difference between dietary intake and exercise energy expenditure, not the stress of exercise. Specifically, Loucks and colleagues' (1994, 1998, and 2003) 3 to 5 days experiments established that LH pulsatility is disrupted abruptly at an energy availability threshold no higher than 30kcal/kgLBM/day (Table 2.4 and Figure 2.1). Congruently, DeSouza et al. (1998) 3-month observations demonstrated that the exercising women with anovulatory (ExANOV) menstrual cycles consumed significantly lower dietary intakes ($p = 0.003$) than exercising women with ovulatory cycles (ExOV) and exercising women with luteal phase deficiencies (ExLPD). As well, the ExANOV group displayed a mean energy availability (EA) of 24.7kcal/kgLBM/day, corresponding with Loucks' EA threshold below 30kcal/kgLBM/day. Conversely, DeSouza's ExOv and ExLPD presented conflicting results, as the ExLPD energy availability of 32.2kcal/kgLBM/day was greater than ExOvul group's EA of 28.7kcal/kgLBM/day. Furthermore, the ExLPD EA of 32.2kcal/kgLBM/day is higher than Loucks and colleagues' threshold of 30kcal/kgLBM/day.

The prevalence of menstrual cycle disturbances with a mean EA control phase of 36.1 +/- 4.3kcal/kgLBM/day did not correspond with the identified EA threshold of below 30kcal/kgLBM/day. Furthermore, dividing the participants according to their menstrual cycle status in the control phase (Table 4.6) reveals that the ROMC (n = 7) group had an EA of 0.3kcal/kgLBM/day higher than the SLP group (n = 6) and 0.8kcal/kgLBM/day higher than the ANOV group (n = 4). Conversely, the ANOV group did follow a similar pattern to DeSouza's et al. (1998) ExANOV group by consuming 234.8 fewer calories per day and displaying a 158.7kcals/day lower EA than the ROMC and SLP groups. This pattern is suggestive that energy availability, regardless of lean body mass, may have played a role in the anovulatory disturbances. The between group conclusions are limited, as a statistical analysis was not performed due to the small sample size. Moreover, it remains unclear whether the intra- and inter- study energy availability incongruencies are attributable to dissimilar methodologies, length of observations, or outcome variables and/or to alternative mechanisms such as exercise stress, gynecological age, or a multi-factorial approach.

It is highly probable that the intra- and inter-study comparisons are ambiguous due to the diverse precisions of the energy availability measurement techniques, as well as, the dissimilar lengths of observations and outcome variables. In particular, Loucks and colleagues (1994, 1998, and 2003) controlled the participants' dietary intakes precisely with clinical dietary products and their compliance was measured by a urinary dipstick assay for ketone acetoacetate. Moreover, Loucks and colleagues monitored the rate of exercise energy expenditure precisely according to the individual's oxygen uptake and respiratory exchange ratio (RER) on a treadmill. Although Loucks and colleagues' techniques are highly precise, the methods are not viable options to monitor energy availabilities over longer observation periods. The only other study to document energy availabilities over a longer duration was DeSouza et al. (1998). She measured 7-day dietary intakes and energy expenditure with the use of a Caltrac accelerometer, which is a motion sensor that estimates energy expenditure. Similarly, our study also used practical methods with a 3-day dietary intake record and approximating the exercise energy expenditure with the compendium of physical activity guide (Ainsworth et al., 2000). The diverse measuring techniques limit the inter-study comparisons. However, the 3-month observations of DeSouza's et al. (1998) and the 2-month observations in the control phase of this study may be more reflective of the chronic effects of a training regimen than Loucks 3- to 5-day experiments. In reference to outcome variables, it is possible that Loucks' LH pulsatility measurements respond

to a different EA threshold than the luteal and ovulatory function, as measured by DeSouza et al. (1998) and our study.

The alternative mechanisms of exercise stress and gynecological age may also have played a role in the menstrual cycle disruptions during the control phase. Table 4.6 highlights that the ANOV group completed a higher mean volume of exercise (109.4 +/- 72.4 TRIMPS/week) as compared to the ROMC group (85.7 +/- 38 TRIMPS/week) and SLP group (84.3 +/- 50.6 TRIMPS/week) over the control phase. Additionally, the ANOV group had a lower mean gynecological age at 15 years past menarche, as compared to the ROMC group at 19.7 years and the SLP group at 20.6 years past menarche. As such, the ANOV participants may have been under more stress with higher training volumes, as well as, more susceptible with a younger gynecological age. It is further plausible that a combination of factors contributed to the menstrual cycle and ovulatory disturbances in the control phase; namely, energy availability, stress of entering the study and of exercise, as well as, gynecological age. It is of note that the ANOV group had a large variability in exercising volumes and gynecological ages.

5.2 Training Program, Energy Availability, and Menstrual Cycle Parameters:

This is the first study to prospectively document energy availability while abruptly increasing the training program in gynecologically mature and recreationally active women. In this study, the menstrual cycle, luteal phase, and follicular phase lengths, as well as, ovulation status were robust to further disturbances regardless of an abrupt increase in training volumes and a decrease in energy availability. Furthermore, the control to training phase difference in luteal phase length was not associated with the changes in energy availability, body composition, or training volume (Figure 4.7). To date, exercise stress investigators have suggested that 2 to 3 month abruptly increasing training program will disturb ovulatory and luteal function (Bullen et al., 1985 and Williams et al., 1999). Menstrual cycle and follicular phase length disturbances have yet to be prospectively observed in recreationally active women. However, these exercise stress findings are limited as the investigators neglected to monitor energy availability. In this study, the menstrual cycle and follicular phase length outcomes are consistent with previous research. However, the luteal phase and ovulatory status outcomes oppose the exercise stress literature and conflict with the energy availability research.

In this study, twenty recreationally active women with an initial aerobic fitness level of 37.6 +/- 3ml/kg/min successfully increased the volume and intensity (measured in TRIMPS) in the first three weeks and in the 5^{th} and 6^{th} weeks of the training program. As illustrated in Table

4.10 and Figure 4.5, the training program increased in the 1st week (15.1%), 2nd week (14.3%), 3rd week (12.1%), 5th week (34.4%), and 6th week (21.7%). As designed, the participants recovered in weeks 4 and 8 by decreasing their training volumes. Participants did not adhere to the 7th week training program increase, as TRIMPS decreased by -16.8%. The five training weeks that increased had a mean rate increase of 19.5% per week. This rate of increase is considered abrupt, as it is recommended to increase gradually at approximately 10% per week. Despite the abrupt increases in TRIMPS, the participants' aerobic capacity and ventilatory threshold only increased slightly over the 8-weeks. Participants significantly increased their ventilatory threshold (VT) by 2 ml/kg/min and total time to VT by 33.3 seconds, and VO_2 peak insignificantly increased by 2.3 ml/kg/min. The 8-weeks of training may not have been of sufficient duration to increase aerobic capacity in relatively fit recreationally active women (VO_2 peak = 37.6 ml/kg/min). The participant's compliance and the effectiveness of the training program are reflected in the significant increase in VT and time to VT. The increase in VT allows a higher absolute and relative exercise intensity to be sustained without the accumulation of blood lactate after training (Jones & Carter, 2000).

The mean rate of increase, 19.5%, over 5-weeks successfully increased the amount of stress from the control to training phase. The amount of stress imposed by the training program was assessed by the total stressor index (TSI) and each stressor index sub-category (MSI, PSI, trSI, MiSI). The training program was significantly stressful medically (MSI), mainly participants experienced more muscle/joint pain in the second training MC as compared to the control and training MC #1. In addition, the participants felt that the training program became more difficult (trSI) in the training phase versus the control phase, especially in the training MC #2. Participants did not feel stressed psychologically (PSI) by the program. And of particular interest, the irregular diets and number of time zones traveled (MiSI) were affecting the participants training less in the training phase versus the control phase. It is unclear whether the participants' training was adapting to their irregular diets and travels or whether the participants were eating more regular diets and traveling less. The irregular diets and travel in the control phase could potentially be a contributing factor to the high prevalence of menstrual cycle disturbances observed in the control phase. Overall, the stress of the training program did increase, as indicated by the total stress index (TSI); although non-significantly.

Although the training program increases abruptly and the total stressor index increased, the absence of luteal and ovulatory changes in the training phase is contrary to the current exercise stress literature. In particular, 66% of Bullen's et al. (1985) weight-maintenance group

experienced abnormal luteal function and 42% became anovulatory. The untrained participants completed a 5-week abruptly increasing training program, 15 to 37.5% per week. Furthermore, forty-five percent of Williams et al. (1999) untrained runners experienced luteal phase shortenings by imposing Bullen's abruptly increasing training program in either the follicular or luteal phase, independently, over 3-months. In our study, the range of training program increases, 12.1 to 34.4% per week, is similar to the rate of increase in Bullen's et al. (1985) and Williams' et al. (1999). Moreover, the increase in aerobic fitness by 2.3 ml/kg/min is congruent with Williams' et al. (1999) increase of 5.55 ml/kg/min, considering Williams' et al. training program had an additional month. Bullen et al. (1985) did not document the changes in aerobic fitness. Conversely to the similar rates of increase, type, duration, frequency, and intensities of training, Bullen's and Williams' participants completed higher training volumes. The participants in this study completed a maximum of 55.2 km per week, as compared to Bullen's 80.5 km per week and Williams' 102.5km over 10 to 20 days. In addition to the higher training volumes, Bullen's and Williams' participants were untrained, regularly ovulatory with normal luteal phase lengths in the control phase, and 10 gynecological years younger than the recreationally active women in this study. As such, the participants in our study may have been more robust to further changes, as menstrual cycle disturbances were already present; the training volumes were lower; the participants were gynecologically mature; and recreationally active prior to entering the study. Another potentially confounding factor is energy availability. However, Bullen et al. (1985) and Williams et al. (1999) neglected to measure energy availability. Our study is the first to document energy availability while abruptly increasing the training volume and intensity in recreationally active women.

Researchers have demonstrated that the mechanism underlying the menstrual cycle disturbances may be energy availability, regardless of the training stress (Loucks et al. 1994, 1998, and 2003; Williams, Caston-Balderrama, et al. 2001; and DeSouza et al., 1998). In this study, the training phase mean energy availability, 31.4kcal/kgLBM/day, decreased by 4.8kcal/kgLBM/day from the control phase with no changes in weight or body composition. The participants' measurements of weekly weight, as well as, the pre- and post- training program measurements in body mass index (kg/m^2), waist to hip ratio, sum of 6 skinfolds, and percent body fat remained constant. The participants also did not modify total caloric and macronutrient intakes before and during the training program. These results concur with the existing literature, as the participants' menstrual cycles were robust to further disruptions while maintaining a mean EA above 30kcal/kgLBM/day. Furthermore, the presence of ovulatory and shortened luteal

phases with a training phase EA of 31.4kcal/kgLBM/day agrees with DeSouza's et al. (1998) observations. She observed ovulatory and luteal phase deficient menstrual cycles in recreationally active women with energy availabilities of 28.7 and 32.2kcal/kgLBM/day, respectively. Conversely, the presence of anovulatory menstrual cycles at an EA above 30kcal/kgLBM/day conflicts with DeSouza's observations. Specifically, DeSouza et al. (1998) observed that anovulatory cycles in recreationally active women occur at a lower mean energy availability of 24.9kcal/kgLBM/day over 3-months. The inter-study comparisons are limited due to the diverse EA measuring techniques, outcome variables, lengths of observation, as discussed previously.

This study broadens the current energy availability and exercise stress research to gynecologically mature and recreationally active women with normal menstrual cycles and menstrual cycle and ovulatory disturbances. Our study also contributes to the existing exercise stress and energy availability literature by revealing that menstrual cycle and ovulatory disturbances are not further disrupted by an abruptly increasing training program when energy availabilities are maintained above 30kcal/kgLBM/day. Future research exploring the notions of energy availability and exercise stress must control the confounding factors of gynecological maturity; presence of menstrual cycle and ovulatory disturbances; and initial activity levels. It is of note that the conclusions cannot be extended to each menstrual cycle group (ovulatory, shortened luteal phases, anovulatory, and oligomenorrheic) separately due to the one-group study design.

5.3 Hormones: The most prominent hormonal changes documented in recreationally active women are decreases in luteal phase progesterone and follicular phase E1C (Beitins et al., 1991; Prior, Vigna, Schechter et al., 1990; Williams et al., 1999; Shangold et al., 1979; Bonen et al., 1981; and DeSouza et al., 1998). As prospectively documented in cynomolgus monkeys, the hormonal disruptions appear to progress from a late luteal phase suppression in the follicular stimulating hormone (FSH) and a decrease in early follicular phase luteinizing hormone (LH) to a decrease in luteal phase progesterone prior to the onset of amenorrhea (Williams, Caston-Balderrama et al., 2001). In this study, there were no significant changes in early follicular phase PdG, luteal phase E1C and PdG, and cortisol from the control to training phase. The control to training phase changes in early follicular phase E1C were approaching significance ($p = 0.012$) and the change in energy availability accounted for 23.1 % of the early follicular phase E1C

change. The hormonal changes, aside from cortisol, are consistent with the current literature in recreationally active women.

Regardless of the amount of energy deficit or strenuous training, the early follicular phase PdG and luteal phase E1C appear to be more robust to changes as compared to luteal phase PdG and early follicular phase E1C. The non-significant control to training phase increase in follicular phase PdG (+709.6 ng/mgCR) and decrease in luteal phase E1C (-4502.6 pg/mgCR) are congruent with the current research, as there have been no studies documenting changes to these hormones in the specific menstrual cycle phases. In contrast, it is well documented that luteal phase PdG is suppressed in recreationally active women (Beitins et al., 1991; Prior, Vigna, Schechter et al., 1990; Williams et al., 1999; Shangold et al., 1979; Bonen et al., 1981; and DeSouza et al., 1998). Incongruently, this study did not show a significant decrease in luteal phase PdG. However, a decrease of 2474.6ng/mgCR was observed from the control to the training phase. The 19.2% decrease is lower than other research findings: Williams, Caston-Balderrama, et al. (2001) observed a 34% decrease in cynomolgus monkeys and Shangold et al. (1979) revealed 50% suppression in a long-distance runner. A further inter-study comparison is not possible, as DeSouza et al. (1998) only observed the prevalence in recreationally active women; Williams, Caston-Balderrama, et al. (2001) documented the changes in cynomolgus monkeys; and other studies did not reported energy availabilities (Beitins et al., 1991; Williams et al., 1999; and Shangold et al., 1979). The contributing factor (s) to the change in luteal phase PdG are unclear, as changes in activity level, energy availability, percent body fat, and weight did not reveal any significant associations. However, current studies reveal that energy deficient cycles are associated with decreases in luteal phase PdG (DeSouza et al., 1998 and Williams, Caston-Balderrama, et al., 2001). Establishing the underlying mechanism is important, as luteal phase PdG is involved in the preparation of the uterine lining for a fertilized egg and has been associated with low cancellous bone mineral density (Prior, Vigna, Schechter, et al., 1990 and Showers, Crutchfield, Bandekar, Randolph, Shapiro, Schork, & Jannausch, 1998). It is of note that the luteal phase E1C and PdG in this study may have been underestimated due to the timing of the urine samples. Specifically, the samples may have been taken when the E1C and PdG values were rising post-ovulation or falling pre-flow.

Researchers have demonstrated inconsistent results with changes in early follicular phase E1C. In particular, DeSouza's et al. (1998) observed a significant suppression in exercising participants with ovulatory, luteal phase deficient, and anovulatory menstrual cycles as compared to sedentary ovulatory participants. Moreover, Loucks & Thuma's (2003) short-term severe

energy restriction resulted in a significant decrease in the mean 24-h pooled estrogen. In contrast, Loucks' less severe energy restrictions, Loucks et al. (1989) observational study in cyclic athletic women, and Prior, Vigna, Schechter, et al.'s (1990) recreationally active runners did not demonstrate any change in E1C levels. The results of this study agree with a mean decrease in follicular phase E1C ($p = 0.012$). In exploring the early follicular phase changes, a large between-group (participants with ROMC in the control phase and participants with subtle menstrual cycle disturbances) difference is observed (Figure 4.11). Specifically, the ROMC group experienced a mean decrease of 17300.9 pg/mgCR; whereas, the participants with subtle menstrual cycle disturbances had a mean decrease of 4030.7 pg/mgCR. Statistical analysis was not performed due to the small sample size. These observations reveal that ovulatory, short luteal phase, and anovulatory menstrual cycles experienced a mean decrease in early follicular E1C; particularly, the ROMC participants. Participants with menstrual cycle lengths greater than 35 day cycles ($n = 3$) were excluded from these observations. A decrease in early follicular phase E1C is suggestive of a decrease in the follicular-stimulating hormone's (FSH) ability to recruit follicles. Moreover, the decrease in early follicular phase E1C is suggestive of a progressive suppression in follicular maturation (DeSouza et al., 1998).

In terms of the underlying mechanism (s), this is the first study to demonstrate that the change in energy availability from the control to training phase accounts for 23.1% of the variance in early follicular phase E1C change. It is of note that changes in exercise, body fat percent, and weight were not associated with early follicular phase E1C changes. In support of the relationship between energy availability and menstrual cycle disturbances, Loucks & Thuma's (2003) demonstrated that LH pulsatility effects begin at a an approximate EA threshold of 30kcal/kgLBM/day and become more extreme as energy availability is further reduced below 20kcal/kgLBM/day (Table 2.4). DeSouza et al. (1998) further observed that the energy availability in exercising anovulatory women was 7.5kcal/kgLBM/day lower than exercising women with luteal phase deficient menstrual cycles. Accordingly, our study revealed that more subtle menstrual cycle disturbances (changes to early follicular phase E1C) occurred with small energy availability changes that remained above 30kcal/kgLBM/day. Although speculative, this inter-study comparison suggests that a dose response relationship may exist between the severity of menstrual cycle disturbances and the amount of energy deficit. Specifically, menstrual cycle disturbances occurring between 26 and 36kcal/kgLBM/day include suppressed early follicular phase E1C and luteal phase PdG, and shortened luteal phases. Disturbances occurring below 25kcal/kgLBM/day include disruptions in LH pulsatility and anovulatory menstrual cycles. It is

of note that other potential contributing factors such as gynecological age and present menstrual cycle disturbances must be taken into consideration.

Researchers have also proposed a complex mechanism between the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-pituitary-gonadal (HPG) axis (Ferin, 1999). As summarized by Ferin (1999), human studies have documented high cortisol levels in association with eating disorders and post-exercise. Moreover, high cortisol levels have been linked with menstrual cycle disturbances in animals (Xiao et al., 2002). However, researchers have neglected to assess energy availability and a direct causal relationship has yet to shown. In this study, cortisol did not significantly increase from the control to the training phase (+5582.4 pg/mgCR); suggesting that the participants were not stressed from the training program or decrease in energy availability. It is of note that only one urine sample was taken during each time period and that cortisol is highly influenced by both exercise stress and sleep/wake cycles. As such, it is possible that the cortisol levels were highly influenced by other factors other than the training program and decreased energy availability (e.g. sleep/wake cycles). Furthermore, the small sample size limits the conclusions, as the change of cortisol was highly influenced by extreme changes (Figure 4.12).

In conclusion, the key hormonal finding in this study is that the change in energy availability accounts for 23.1% of the variability in early follicular phase E1C change. This finding strengthens the current energy availability research by adding to the hormonal and ovulatory disturbances associated with energy deficits. This study also broadens the current energy availability and hormonal disturbances research to gynecologically mature and recreationally active women with normal menstrual cycles and menstrual cycle and ovulatory disturbances. Additional research exploring the potential energy availability relationship is needed in gynecologically mature women. Furthermore, future research needs to prospectively explore the potential dose-response relationship between the severity of menstrual cycle, ovulatory, and hormonal disturbances and the amount of energy deficit.

5.4 Other findings: The change in pre-menstrual symptoms, the recruitment of participants and number of drop-outs, as well as injuries were described in the study.

5.4.1 Pre-Menstrual Symptoms: The pre-menstrual symptoms of breast size, front and side breast soreness, energy, fluid retention, moods (anxious, depression, and frustration), and appetite did not change throughout the study. In contrast, Prior & Vigna's (1987) regularly

ovulatory marathon runners (n = 7) decreased in fluid and depression symptoms, and to some extent anxiety after training for 6-months. The main differences between our study and Prior's marathon group include the 2- versus 6-month observation period; Prior & Vigna's participants were normally ovulatory prior the 6-months training program; and Prior & Vigna's analyzed the last 14-days of the menstrual cycles versus the last 7 days in this study. As such, decreases in pre-menstrual symptoms may have occurred with a training program longer than 2-months. It is also plausible that the participants in our study had previously adapted to the effects of the exercise, given the presence of menstrual cycle disturbances in the control phase.

5.4.2 Participant recruitment: The recruitment of participants was difficult, as only 8% of the 250 volunteers completed the study. In particular, 205 (82%) candidates were excluded based on the study's criteria, which corresponds to Prior, Vigna, Schechter et al.'s (1990) exclusion of 132 (54%) candidates based on similar criteria. The higher exclusion rate in this study can be explained by the added activity level criteria. The main reasons for exclusion were high volumes of activity, changes in weight, and an insufficient amount of time. In terms of drop-outs, 68% discontinued in the control phase and 32% did not complete the training phase. The main reason for the drop-outs was due to time constraints. The 32% drop-out during the training phase is consistent with other training studies, as the dropout rate for adult fitness programs is approximately 25 to 35 % across 10-20 weeks (Pollock, 1988). Furthermore, the primary reason for the 22% drop-outs in Branch, Russell, and Bourque's (2000)12-week gradually increasing training program was a lack of time. The adherence to a training program is reported to increase when intensity is 50% of aerobic capacity or lower (Dishman and Buckworth, 1996).

5.4.3 Injuries: There were no documented injuries throughout the study. However, participants did experience an increase in muscle and joint pain from the control to the training phases. During various 13-week running clinics, the incidence of injuries was reported as 29.5% and tibial stress syndrome was the most commonly diagnosed injury (Taunton, Ryan, Clement, McKenzie, Lloyd-Smith, and Zumbo, 2003). In Taunton's et al. (2003) study, the reported injuries were at least grade 1; pain only after running. No reports of injuries in this study may be a result of the short duration of the training program (8-weeks), the participants' initial fitness levels, and the combination of running and low-impact exercises (cross-training).

5.5 Summary: In summary, recreationally active women have a high prevalence of menstrual cycle and ovulatory disturbances. The stress of an 8-week abruptly increasing training program had no effects on the menstrual cycle, follicular phase, and luteal phase lengths, as well as, luteal and follicular phase hormones. A change in energy availability by 4.8kcal/kgLBM/day accounted for 23.1% of the variance in early follicular phase E1C change. To confirm the contributing factors of menstrual cycle and hormonal effects in recreationally active women in long-term studies, investigators must establish accurate and precise means to evaluate energy availability. The energy availability methods must also be practical and inexpensive. Future research considerations should include short- and long-term studies attempting to establish the dose-response relationship between the severity of energy availability and the severity of menstrual cycle disturbances. In addition, researchers must explore other possible confounders such as gynecological age and the presence of menstrual cycle disturbances. Future studies also need to explore means of reversing the menstrual cycle disturbances and establish specific energy availability guidelines to maintain reproductive health in recreationally active women.

References

Ainsworth, B.; Haskell, W.; Whitt, M.; Irwin, M.; Swartz, A.; Strath, S.; O'Brien, W.; Bassett, D.; Schmitz, K.; Emplaincourt, P.; Jacobs, D.; Leon, A. (2000). Compendium of Physical Activities: an update of activity codes and MET intensities. Medicine & Science in Sports Exercise. 32 (9): S498 – S516.

Assay Designs Inc. (2004). Casey Frushour and Josef Guzowski (eds.). Retrieved 1 June 2005, <http://www.assaydesigns.com/products/catalog/immuno_assay/product_cortisol.htm>.

Banister, E.W. and Hamilton, C.L. (1985). Variations in iron status with fatigue modelled from training in female distance runners. European Journal of Applied Physiology. 54: 16 – 23.

Barr, S.I. & Prior, J.C. (1994). The menstrual cycle: effects on bone in premenopausal women. Advances in Nutritional Research. 9: 287-310.

Beitins, I.Z., McArthur, J.W., Turnball, B.A., Skrinar, G.S., & Bullen, B.A. (1991). Exercise induces two types of human luteal dysfunction: Confirmation of urinary free progesterone. Journal of Clinical Endocrinology and Metabolism. 72: 1350 – 1358.

Boden, G., Chen, X., Mozzoli, M., & Ryan, I. (1996). Effect of fasting on serum leptin in normal human subjects. Journal of Clinical Endocrinology and Metabolism. 81: 3419-3423.

Bonen, A. (1992). Recreational exercise does not impair menstrual cycles: a prospective study. International Journal of Sport Medicine. 13(2): 110-120.

Bonen, A., Belcastro, A.N., Ling, W.Y., & Simpson, A.A. (1981). Profiles of selected hormones during menstrual cycles of teenage athletes. Journal of Applied Physiology: Respiratory, Environment, and Exercise Physiology. 50 (3): 545 – 551.

Boyden, T.W., Pamenter, R.W., Grosso, D., Stanforth, P., Rotkis, T., & Wilmore, J.H. (1982). Prolactin responses, menstrual cycles, and body composition of women runners. Journal of Clinical Endocrinology & Metabolism. 54:711-713.

Branch J.D., Russell R.P., and Bourque, S.P. (2000). Moderate intensity exercise training improves cardiorespiratory fitness in women. Journal of Women Health & Gender-Based Medicine. 9(1): 65 – 73.

Bullen, B.A., Skrinar, D.G., Beitins, I.Z., Gretchen von Mering, B.S., Turnbull, B.A., & McArthur, J.W. (1985) Induction of menstrual disorders by strenuous exercise in untrained women. The New England Journal of Medicine. 312(21): 1349 –1353.

DeSouza, M.C., Walker, A.F., Robinson, P.A., Bolland, K. (2000). A synergistic effect of a daily supplement for 1 month of 200mg Magnesium plus 50mg vitamin B_6 for the relief of anxiety-related premenstrual symptoms: a randomized, double-blind, crossover study. Journal of Women's Health & Gender-Based Medicine. 9(2): 131-139.

DeSouza, M.J. (2003). Menstrual disturbances in athletes: a focus on luteal phase defects. Medicine & Science in Sports & Exercise. 35(9): 1553-1563.

DeSouza, M.J., Leidy, H.J., O'Donnell, E., Lasley, B. & Williams, N.I. (2004). Fasting ghrelin levels in physically active women: relationship with menstrual disturbances and metabolic hormones. The Journal of Clinical Endocrinology & Metabolism. 89(7): 3536-3542.

DeSouza, M.J. & Metzger, D.A. (1991). Reproductive dysfunction in amenorrheic athletes and anorexic patients: a review. Medicine & Science in Sports & Exercise. 23(9): 995-1007.

DeSouza, M.J., Miller, B.E., Loucks, A.B., Luciano, A.A., Pescatello, L.S., Campbell, C.G., & Lasley, B.L. (1998). High frequency of luteal phase deficiency and anovulation in recreational women runners: blunted elevation in follicular – stimulating hormone observed during luteal – follicular transition. Journal of Clinical Endocrinology and Metabolism. 83: 4220-4232.

DeSouza, M.J., Miller, B.E., Squenzia, L.C., Luciano, A.A., Ulreich, S., Stier, S., Prestwood, K., & Lasley, B.L. (1997). Bone health is not affected by luteal phase abnormalities and decreased ovarian progesterone production in female runners. Journal of Clinical Endocrinology and Metabolism. 82: 2867-2876.

DeSouza, M.J., Van Heest, J., Demers, L.M., & Lasley, B.L. (2003). Luteal phase deficiency in recreational runners: evidence for a hypometabolic state. The Journal of Endocrinology & Metabolism. 88(1): 337-346.

Dishman and Buckworth (1996). Increasing physical activity: a quantitative synthesis. Medicine and Science in Sports and Exercise. 28(6): 706-719.

Ferin, M. (1999). Clinical review 105: Stress and the reproductive cycle. The Journal of Clinical Endocrinology and Metabolism. 84(6): 1768 – 1774.

Fry, R.W., Morton, A.R., & Keast, D. (1992) Periodisation and the prevention of overtraining. Canadian Journal of Sport Science. 17(3): 241-248

Hilton, L.K. & Loucks, A.B. (2000). Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. American Journal of Physiology, Endocrinology, and Metabolism. 278: E43-49.

Ihle, R. & Loucks, A.B. (2004). Dose-response relationship between energy availability and bone turnover in young exercising women. Journal of Bone and Mineral Research. 19(8): 1231-1240.

Jackson A.S. & Pollock M.L. (1985). Practical assessment of body composition. Physicians and Sports Medicine. 13: 76-90.

Jones, A.M. & Carter, H. (2000). The effect of endurance training on parameters of aerobic fitness. Sports Medicine. 29(6): 373-386.

Loucks, A.B. & Heath, E.M. (1994). Dietary restriction reduces luteinizing hormone (LH) pulse frequency during waking hours and increases LH pulse amplitude during sleep in young menstruating women. Journal of Clinical Endocrinology & Metabolism. 78(4): 910-915.

Loucks A.B. & Horvath, S.M. (1985). Athletic amenorrhea: a review. Medicine, Science, and Sports Exercise. 17(1): 56-72.

Loucks A.B. & Horvath, S.M. (1984). Exercise-induced stress responses of amenorrheic and eumenorrheic runners. Journal of Clinical Endocrinology and Metabolism. 59: 1109 – 1120.

Loucks, A.B., Horvath, S.M., and Freedson, P.S. (1984). Menstrual status and validation of body fat prediction in athletes. Human Biology. 56(2): 383-392.

Loucks, A.B., Laughlin, G.A., Mortola, J.F., Girton, L., Nelson, J.C., & Yen, S.C. (1992). Hypothalamic-Pituitary-Thyroidal function in eumenorrheic and amenorrheic athletes. Journal of Clinical Endocrinology and Metabolism. 75: 514 – 518.

Loucks, A.B., Mortola, J.F., Girton, L., & Yen, S.S.C. (1989). Alterations in the Hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. Journal of Clinical Endocrinology and Metabolism. 68: 402 – 411.

Loucks, A.B. & Redman, L.M. (2004). The effect of stress on menstrual function. Trends in Endocrinology and Metabolism. 15(10): 466-471.

Loucks, A.B. & Thuma, J.R. (2003). Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. The Journal of Clinical Endocrinology & Metabolism. 88(1): 297-311.

Loucks, A.B. & Verdun, M. (1998). Slow restoration of LH pulsatility by refeeding in energetically disrupted women. Regulatory, Integrative, and Comparative Physiology. 44: R1218-R1226.

Loucks, A.B., Verdun, M., & Heath, E.M. (1998). Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. Journal of Applied Physiology. 84(1): 37-46.

McArthur, J.W. (1985). Endorphins and exercise in females: possible connection with reproduction dysfunction. Medicine & Science in Sports & Exercise. 17(1): 82-88.

McConnell, H.J., O'Connor, K.A., Brindle, E., & Williams, N.I. (2002). Validity of methods for analyzing urinary steroid data to detect ovulation in athletes. Medicine and Science in Sports and Exercise. 34(11): 1836-1844.

McKenzie, D.C. (1995). Overtraining. In Torg J.S. and Shephard R.J., Current Therapy in Sports Medicine 3rd edition. (pp.526 – 530). USA: Mosby-Year Book Inc.

McLean, J.A., Barr, S.I., & Prior, J.C. (2001). Dietary restraint, exercise, and bone density in young women: are they related? Medicine Science Sports and Exercise. 33(8): 1292 – 1296.

Nutritionist Five Version 1.6 (1998) First Databank, Inc., San Bruno, California.

O'Connor, K.A., Brindle, E., Holman, D.J., Klein, N.A., Soules, M.R., Campbell K.L., Kohen, F., Munro, C.J., Shofer, J.B., Lasley, B.L., & Wood, J.W. (2003). Urinary Estrone Conjugate and Pregnanediol 3-Glucuronide Enzyme Immunoassays for Population Research. Clinical Chemistry. 49(7): 1139-1148.

Petite M.A. & Prior J.C. (2000). Exercise and the hypothalamus: ovulatory adaptations. In Warren, M. P. and Constantini, N.W. (eds.). Sports Endocrinology. New Jersey. Human Press.

Petit, M.A., Prior, J.C., and Barr, S.I. (1999). Running and ovulation positively change cancellous bone in premenopausal women. Medicine and Science in Sports and Exercise. 31(6): 780-787.

Pollock, M.L. (1988). Prescribing exercise for fitness and adherence. In Dishman R.K. (Ed.). Exercise Adherence: Its Impact on Public Health (pg. 259 – 277). Champaign, IL. Human kinetics.

Prior, J.C. (1996a). The cultural and historical perspective etiology: adaptation as a fundamental physiologic process. In Adashi, J.A., Rock, J.A., and Rosenwaks, Z. (Eds.), Reproductive Endocrinology. (pg. 1078 – 1090). Philedelphia. Lippincott – Raven Publishers.

Prior, J.C. (1996b). High dose medroxyprogesterone, rates of bone resorption, and changes in spinal bone density. Journal of Clinical Endocrinology and Metabolism. 81(12): 4500 – 4501.

Prior, J.C. (1997). Ovulatory disturbances: they do matter. The Canadian Journal of Diagnosis.69 –80.

Prior, J.C., Cameron, K., HoYuen, B.H., & Thomas, J. (1982). Menstrual cycle changes with marathon training: Anovulation and short luteal phase. Canadian Journal of Applied Sport Science. 7(3): 173 – 177.

Prior, J.C., Yuen, B.H., Clement, P., Bowie, L., & Thomas, J (1982). Reversible luteal phase changes and infertility associated with marathon training. The Lancet. 1: 269-270.

Prior, J.C. &Vigna Y. (1987) Conditioning Exercise and Pre-menstrual Symptoms. Journal of Reproductive Medicine. 32: 423 – 428.

Prior, J.C., Vigna, Y.M., Barr, S.I., & Lentle, B.C. (1994). Cyclic medroxyprogesterone treatment increases bone density: a controlled trial in active women with menstrual cycle disturbances. The American Journal of Medicine. 96: 521-530.

Prior, J.C., Vigna, Y.M., Schulzer, M., Hall, J.E., & Bonen, A. (1990). Determination of luteal phase length by quantitative basal temperature methods: Validation against the midcycle LH peak. Clinical and Investigative Medicine.13 (3): 123-131.

Prior J.C., Vigna, Y.M., Schechter, M.T., & Burgess, A.E. (1990) Spinal bone loss and ovulatory disturbances. The New England Journal of Medicine. 3232(18): 1221 – 1227.

Rickenlund, A., Carlstrom, K., Bjorn, E., Brismar, T., von Schoultz, B., & Hirschberg, A. (2003). Hyperandrogenicity is an alternative mechanism underlying oligomenorrhea or amenorrhea in female athletes and may improve physical performance. Fertility and Sterility. 79(4): 947 – 955

Rogol, A.D., Weltman, A., Weltman, J.Y, Seip, R.L., Snead, D.B., Levine, S., Haskvitz, E. M., Thompson, D.L., Schurrer, R., Dowling, E., Walberg – Rankin, J., Williams, E., & Veldhuis, J. (1992). Durability of the reproductive axis in eumenorrheic women during 1 yr of endurance training. Journal of Applied Physiology. 72(4): 1571 – 1580.

Seyle, H. (1939). The effect of adaptation to various damaging agents on the female sex organs in the rat. Endocrinology. 25: 615-624.

Shangold, M.M., Freedman, B., Thyssen, B., & Mengold, G. (1979). The relationship between long distance running, plasma progesterone, and luteal phase length. Fertility and Sterility. 31: 130 – 137.

Showers, M-F, Crutchfield, M., Bandekar, R., Randolph, J.F., Shapiro, B., Schork, M.A., & Jannausch, M. (1998). Bone mineral density and its change in pre- and perimenopausal white women: the Michigan bone health study. Journal of Bone and Mineral Research. 13(7): 1134 – 1140.

SPSS Inc. (2003). SPSS Base 12.0 for Window. SPSS Inc., Chicago IL.

Stunkard, A. J. and Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. Journal of Psychosomatic Research 29: 71-83.

Taunton, J.E., Ryan, M.B., Clement, D.B., McKenzie, D.C., Lloyd-Smith, D.R., Zumbo, B.D. (2003). A prospective study of running injuries: the Vancouver Sun Run "In Training" clinics. British Journal of Sports Medicine. 37: 239-244.

Taussky HH (1954) A microcolorimetric determination of creatinine in urine by the Jaffe reaction. Journal of Biological Chemistry 208: 853-861.

Thomas, S., Reading, J., and Shepard, R.J. (1992). Review of the physical activity readiness questionnaire (PAR-Q). Canadian Journal of Sport Science. 17(4): 338-345.

Torstveit, M.K. & Sundgot-Borgen, J. (2005). Participation in leanness sports but not training volume is associated with menstrual dysfunction: a national survey of 1276 elite athletes and controls. British Journal of Sports Medicine. 39: 141-147.

Volkov, N.I., Shirkovets, E.A., Borilkevich, V.E. (1975). Assessment of aerobic and anaerobic capacity of athletes in treadmill running tests. European Journal of Applied Physiology and Occupational Physiology. 34(2): 121-130.

Vollman (1977). The Menstrual Cycle. Philedelphia, London, and Toronto. W. B. Saunders Company.

Wade, G.N. & Jones, J.E. (2003). Lessons from experimental disruption of estrous cycles and behaviors. Medicine & Science in Sports & Exercise. 35(9): 1573-1580.

Wade, G.N., Schneirder, J.E., & Li, H.Y. (1996). Control of fertility by metabolic cues. American Journal of Physiology. 270: E1-E19.

Williams N.I. (2003). Lessons from experimental disruptions of the menstrual cycle in humans and monkeys. Medicine & Science in Sports & Exercise. 35(9): 1564-1572.

Williams, N.I., Bullen, B.A., McArthur, J.W., Skrinar, G.S., & Turnball, B.A. (1999). Effects of short – term strenuous endurance exercise upon corpus luteum function. Medicine and Science in Sports and Exercise. 31(7): 949 – 958.

Williams, N.I., Caston-Balderrama, A.L., Helmreich, D.L, Parfitt, D.B., Nosbisch, C., & Cameron, J.L. (2001). Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: abrupt transition to exercise-induced amenorrhea. Endocrinology. 142: 2381-2389.

Williams, N.I., Helmreich, D.L, Parfitt, D.B., Caston-Balderrama A., & Cameron, J.L. (2001). Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. The Journal of Clinical Endocrinology & Metabolism. 86(11): 5184-5193.

Williams, N.I., Lancas, M.J., & Cameron, J.L. (1996). Stimulation of luteinizing hormone secretion by food intake: evidence against a role for insulin. Endocrinology. 137(6): 2565-2571.

Williams, N.I.; McArthur, J.W.; Turnball, B.A.; Bullen, B.A.; Skrinar, G.S.; Beitins, I.Z.; Besser, G.M.; Rees, L.H.; Gilbert, I.; Cramer, D.; Perry, L.; and Tunstall Pedoe, D.S. (1994). Effects of follicular phase exercise on luteinizing hormone pulse characteristics in sedentary eumenorrhoeic women. Clinical Endocrinology. 41: 787-794.

Williams, N.I., Young, J.C., McArthur, J.W., Bullen, B., Skrinar, G.S., and Turnbull, B. (1995). strenuous exercise with caloric restriction: effect on luteinizing hormone secretion. Medicine & Science in Sports & Exercise. 27(10): 1390-1398.

Xiao, E., Xia-Zhang, L., & Ferin, M (2002). Inadequate luteal function is the initial clinical cyclic defect in a 12-day stress model that includes a psychogenic component in the rhesus monkey. Journal of Clinical endocrinology & Metabolism. 87: 2242-2237.

Appendix A: Menstrual Cycle Diary

Subject Number: Year: _____

Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Date																															
Tampons/pads/day																															

Record 0 = none, 1 = minimal, 2 = moderate, 3 = moderately intense, 4 = very intense

Amount Flow																															
Cramps																															
Breast Sore: Front																															
Breast Sore: Side																															
Fluid Retention																															
Mucous secretions																															
Constipation																															
Headache																															
Sleep Problems																															
Feeling Frustrated																															
Feeling Depressed																															
Feeling Anxious																															

Record M = much less, L = a little less, U = usual, Y = a little increased, Z = much increased

Appetite																															
Breast Size																															
Interest In Sex																															
Feeling Of Energy																															
Feeling Of Self-Worth																															
Outside Stresses																															
Basal Temperature																															
Dates of Urine samples																															
Comments (temperature taken late, feeling sick, poor sleep, etc)																															

Definitions of Follicular and Luteal Menstrual Cycle Phases

Follicular Phase: Counting from the first day of your menstrual cycle (1st day of menses), your follicular phase urine sample must be taken between Days **2 and 5.**
Luteal Phase: Luteal Phase urine samples must be taken approximately 6 days before the end of your menstrual cycle.
HOW? There are three methods for the timing of luteal phase urine collections.

1) If **stretchy mucus** appears mid-cycle, please take urine sample 3-days after stretchy mucus has gone away.
2) If your **temperature** rises for 3-5 days mid-cycle (36.7°), please take urine sample.

OR IF ABOVE FAILS

3) Please take urine sample on day 21.

- If your menses does not start **7 days** after you took your urine sample on day 21, please take another sample on day 28.
- **Please keep taking urine samples at 7-day intervals until your menses begins.**

Your Timing

	2nd "Control" Menstrual Cycle		1st Exercising Menstrual cycle	2nd Exercising Menstrual Cycle
Menstrual Cycle Phases	Follicular	Luteal	Luteal	Follicular
Dates				

III. Urine Collection Instructions

When to Collect Urine
It is important to collect the urine samples during the specified time periods and preferably your first urination after getting up in the morning. We realize that you may urinate during the night or early morning, but we prefer that you wait to collect urine until you're finally up for the day. If some other time of day is best for your routine, please go ahead. All we really need is a *daily* sample. If you do adopt a routine other than a first morning collection, please note it on urine sample label and in your Menstrual Cycle Diary, under the comments section.
What if You Miss a Urine Collection?
If you miss the first urine of the day, collect a later one if you can.
If you do miss the day, please be take urine sample on the following day.

How to Collect Urine

1. Before you urinate in the morning, get a urine collection vial. (It is handy to keep the Ziploc bag of collection vials in the bathroom closet or cupboard.)
2. Unscrew the cap of the vial and remove the cap with attached sponge from the vial (See Figure 1). (Do not remove the sponge from the cap! The cap is designed to be a holder for the sponge).
3. When you sit on the toilet to urinate, hold the top of the cap with your hand, and position the sponge directly in your urine stream. You will notice that sponge expand greatly. Saturate the sponge well, until urine has "wicked" up the sponge and the sponge has expanded to fill the cap (See Figure 2); this should take about 8-12 seconds.
4. After the sponge is saturated, you may wish to wait two or three seconds to prevent dripping.
5. Insert the sponge back into the collection vial and ***screw the cap on tightly.***
6. Complete the information on the urine sample label (calendar date, menstrual cycle day, comments) and attach it firmly around the vial.
7. Place the labeled vial into your container (Ziploc back) in your freezer.
8. Make any appropriate notations on the Menstrual Cycle Diary.
9. If the sponge falls out of the cap and into the toilet or onto the ground, please discard it. Try to get another sample (then or later) with a new urine collection vial.

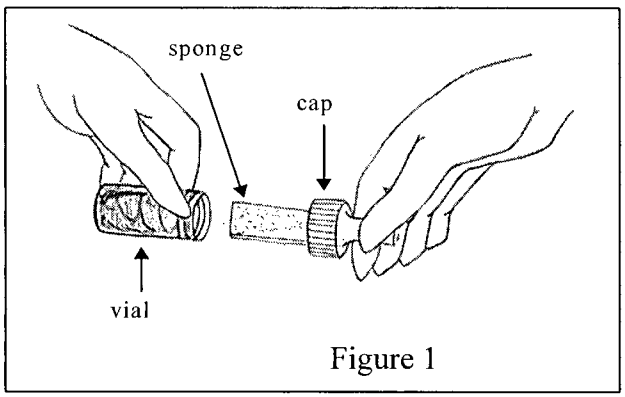
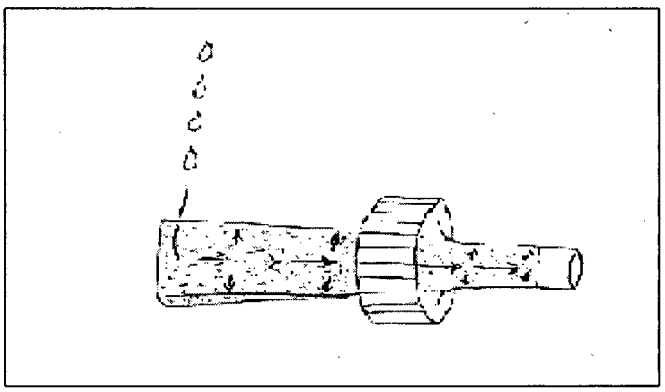


Figure 1



What to Do While Traveling

If you will be traveling, you can collect the sample while you are away from home. You must bring a small cooler and cold pack to use while traveling. Urine collection is easily accommodated while visiting relatives, staying in hotels or even camping (even wilderness camping as some participants have done!).

1. Put the cold pack in your freezer at least one day before traveling.
2. Right before leaving, place the cold pack in the cooler.
3. Remember to take your vial for the duration of your trip.
4. Place your collected sample in the cooler and try to keep them as cool as possible by using a freezer, ice, or a refrigerator at your travel destination. We realize (from experience) that it is sometimes hard to keep samples cold while traveling, and although it is not ideal, it happens that some urine samples end up being stored at room temperature for a day or two. ***Please note on your menstrual cycle diary, if any of your samples have been at room temperature for more than a few hours, and please let us know for approximately how long they were at room temperature.***

IV. How to Store Your Samples

Your urine samples should be kept frozen or as cold as soon as possible at all times — Keeping samples frozen or cold is important for preserving the reproductive hormones in the urine.

You may store samples in your freezer in any type of container such as a plastic bag or any other container that is convenient for you.

It may occasionally happen that you leave a collection vial with a urine sample on the bathroom counter all day, and only put it in the freezer later in the day. This is okay, but try to get the sample into the freezer as soon as possible. ***If this does happen, please make a note of it on your Menstrual Cycle Diary.***

The collection vials seal tightly *(as long as the caps are screwed on tightly)*, so there should be no problem with leaking or spilling of urine in your freezer. A major advantage of the sponges is that the urine is held in the sponge, making leakage highly unlikely.

To prevent drying out of sponges (and loss of urine sample), please make sure the caps of the vials are sealed tightly.

V. Record Keeping and Labeling Instructions

Label Records: Description and Purpose

Your Label Records on the vials contain 1) Your code, and 2) Calendar Date, 3) Menstrual Cycle Day, 4) Menstrual Cycle Phase, 5) Comments.

After you collect your urine, you will need to complete the label on the collection vial. As well, please record the timing of your urine sample on your Menstrual Cycle Diary by marking an "x" in the date of urine sample box. Record any relevant notations corresponding to each urine collection, both on the label and in the Menstrual Cycle Diary. Please use a ballpoint pen or pencil to enter any necessary information.

Write down comments, if any, in the COMMENTS Section of the Menstrual Cycle Diary. In this section we are primarily interested in anything that might 1) alter your body's natural reproductive hormone levels, or 2) affect the quality of a urine sample. We ask that you ***please record any of the items that apply to you.***

- Record if you missed collecting a urine sample from a specified time period (we need to know this for our record keeping–so we know that we have not misplaced one of your samples).
- Record if a urine sample was kept at room temperature or unfrozen for more than a few hours, and note approximately how long it was at room temperature.
- Record if you have become pregnant or are breastfeeding.
- ***Record whether you started or stopped using any prescription or nonprescription medicines or nutritional supplements.*** You can list these items daily, or you can list them once each month at the top of the Menstrual Cycle Diary—this way you don't have to make daily notations. For succeeding months, if there are no changes in your routine, simply write, "Same as Previous Month" so that you do not have to list your items again. But ***please remember to note any deviations from the routine***, or when you stop taking an item, or start a new item.
- Please make sure you convey to us the name of each product you take.
- Record whether you have received any radiation treatment, infertility treatment, a hysterectomy, oophorectomy, or any major surgery.
- If you have nothing to record for a day just leave the Comments section blank. If you have nothing at all to record for several days or a whole month, please write "Nothing to Record" and draw an arrow to indicate the relevant days of nothing to record.

Product List for Comments Section

Below are examples of the *types* of products you should record in the COMMENTS Section.

Prescription Medications

List all prescribed medications you are taking, including those for heart conditions, diabetes, depression, thyroid conditions, infections (antibiotics), etc.

Non-Prescription Hormones, Products and Nutritional Supplements

List all non-prescription substances you take, including vitamins, natural progesterone cream, wild yam cream, supplements, flaxseed, phytoestrogen supplements, herbs, and all other over-the-counter preparations. These include aspirin, Tylenol, ginseng, St. John's Wort, soy supplements, etc.

Extra Labels

Extra labels are included. These are provided in case one of your regular labels gets damaged. If you need to use one of these extra labels, please complete with ballpoint pen or pencil.

Weekly Training Log

Study Number: _____ Month/Week: Weight (**weekly**):

NOTE: Heart rate (HR)
1)10 minutes into exercise 2) half way into exercise 3) at the end of exercise.

Day: **Menstrual Day:**

Workout/Comments	Total Km	Time	Heart Rate
			1 2 3

Day: **Menstrual Day:**

Workout/Comments	Total Km	Time	Heart Rates
			1 2 3

Day: **Menstrual Day:**

Workout/Comments	Total Km	Time	Heart Rate
			1 2 3

Day: **Menstrual Day:**

Workout/Comments	Total Km	Time	Heart Rate
			1 2 3

Day: **Menstrual Day:**

Workout/Comments	Total Km	Time	Heart Rate
			1 2 3

Day: **Menstrual Day:**

Workout/Comments	Total Km	Time	Heart Rate
			1 2 3

Training Monitoring Form

Subject Number:_____ Date:_____

Mark the point (x) in the box that best describes your status at this moment!

Relative to Yesterday

Physical Feelings	**None or Minimal**	**Better**		**Worse**	**Unable to Train**
1. General Fatigue	0	1	2	3	4
2. Muscle/Joint Pain	0	1	2	3	4
3. Sore throat, fever, cough, etc.	0	1	2	3	4
4. Change in Sleep pattern	0	1	2	3	4

Questions	**Positive Very High**		**Average**		**Very Low Consistent Problems**
5. How do you feel about yourself (confidence in training, ability to focus, level of annoyance and frustration)?	0	1	2	3	4
6. How do you feel about your running (satisfaction with training and relationships with individuals inside of running; other runners, researchers, etc.)?	0	1	2	3	4
7. How do you feel about situations outside your running (work, school, $, individuals not involved in running: significant others, etc.)?	0	1	2	3	4

Question	**Easy**		**Average**		**Hard**
8. How does your training program feel	0	1	2	3	4

Questions	**NO**		**Yes, No Affect**		**Yes Affects Running**
9. Irregular diet	0	1	2	3	4
10. Time zones traveled (#)	0	1	2	3	4

Three-day Dietary Intake Guidelines **Please read carefully to maximize accuracy**

An accurately completed dietary intake record can provide valuable information about the nutritional content of an individual's usual diet. Please try and maintain your normal eating patterns in terms of content and quantity of foods consumed during this 3-day period.
Please keep record of everything you eat or drink on the attached forms for three days in a row (including 1 weekend day). Please be as specific and as detailed as possible.

- To ensure accuracy please try to record immediately after eating.
- The more accurately you record, the more meaningful is the analysis!

Be sure to include:

1. ALL FOODS AND DRINKS consumed including snacks, soft drinks, alcohol, cream and sugar in coffee/tea, butter/sauces on vegetables, jams, relishes, candies, butter/margarine/mayonnaise on sandwiches, salad dressing. Break combination foods down into their constituents (e.g. ham and cheese omelette = 3 eggs + 1 oz. Cheddar cheese + 1 slice Oscar Meyer Packaged ham slices + 1 tsp butter in pan)

2. THE AMOUNT OF FOOD that was consumed. It is extremely important for assessment purposes that accurate measurements be recorded. It may be helpful to measure the volume of your regular glasses, bowls and cups before you begin.

- Use VOLUME measures such as cups, tablespoons (tbs.), teaspoons (tsp.) or milliliters (mls) for soup, pasta, cereals, rice, other grains, small or cut vegetables, cut fruit, tinned foods, drinks, sauces, salad dressings, butter, mayonnaise, margarine, jams, peanut butter etc. Please be as accurate as possible. For example, record if a tablespoon is 'heaping' as opposed to 'level'.
- Use WEIGHTS (ounces or grams) OR make it in relation to a "Deck of Cards" for meat, fish, poultry, cheese etc IE: a chicken breast as 1.5 decks of cards. (1 deck = 3 oz) so = 4.5 oz of chicken. Use the labels on packages to help you. If you are dining out, record the size of the piece of the meat e.g. sirloin steak 3" by 4" by ½", or hamburger patty 3" diameter by ½".
- Use SIZES for whole fruits, whole vegetables, cookies, cakes, eggs, cheese pieces or meat etc. Either specify small, medium or large or give dimensions. In some cases it may be more appropriate to give size in relation to a whole. E.g. ½ medium pepperoni pizza, piece of cheddar cheese 2" by 3" by 1", 1 small apple, 1 large bran muffin.

3. THE BRAND NAMES OR TYPE OF FOOD.
   Examples:
   - Sunrise soft tofu - 1/2 cup
   - Benny's whole wheat bagel - 1
   - 1% milk - 1.5 cups
   - Oreo cookies - 3

4. THE TIME OF DAY the foods and beverages were consumed.

5. THE TYPE AND TIME OF EXERCISE/TRAINING ON THESE 3 DAYS - this will help to identify appropriate pre and post-exercise meals for optimizing your workouts/performance.

Day 1 of <u>Three-day Food Record Forms</u> (same forms for Day 2 & Day 3)

Date	Time	Complete Description of Food or Beverage	Portion Size

Page 2 of **Day 1 Three-day Food Record Forms**

Date	Time	Complete Description of Food or Beverage	Portion Size

Was this a typical day for you? Yes _____ No _____

If not please give reason(s):

Appendix B
Training Program in Meters

1st Month

1st week – 20 km

Day	Workouts	Km Run
Monday	VO_2max test and skinfolds	> 1.6 km
Tuesday	Off	0
Wednesday	3 * (1200m above VT, 800m below VT)	6.0 km
Thursday	2 * (1000m above VT, 800m @VT, 800m below VT)	5.2 km
Friday	Off	0
Saturday	3 * (1400m @ VT, 1000m below VT)	7.2 km
Sunday	Off	0

2nd week – 24.5 km

Monday	3 * (1600m above VT, 800m below VT)	7.2 km
Tuesday	Off	0
Wednesday	2 * (1000m above VT, 1000m @ VT, 400m below VT)	4.8 km
Thursday	7.4 km below VT	7.4 km
Friday	Off	0
Saturday	5.1 Km @ VT	5.1 km
Sunday	Off	0

3rd week – 30.0 km

Monday	3 * (1750m above VT, 800m below VT)	7.65 km
Tuesday	5.0 km below VT	5.0 km
Wednesday	Off	0
Thursday	3 * (1000m above VT, 800m @ VT, 400m below VT)	6.6 km
Friday	4.5 km below VT	4.5 km
Saturday	6.3 @ VT	6.3 km
Sunday	Off	0

4th week - Recovery @ 15 km

Monday	Off	0
Tuesday	2 * (2000m above VT, 1000m below VT)	6.0 Km
Wednesday	Off	0
Thursday	4.5 km below VT	4.5 km
Friday	Off	0
Saturday	4.5 Km @ VT	4.5 km
Sunday	Off	0

2nd Month

5th week – 36.8 km

Day	Workouts	Km run
Monday	3 * (1800m above VT, 1000m below VT)	8.4 km
Tuesday	7.5 km @ VT	7.5 km
Wednesday	8.5 km below VT	8.5 km
Thursday	Off	0
Friday	4 * (800m above VT, 800m below VT)	6.4 km
Saturday	Off	0
Sunday	6 km Fartlek – (1.5 km above VT, 3.2 km @ VT, 1.3 km below VT)	6.0 km

6th week – 45.0 km

Monday	3 * (2000m above VT, 1200m below VT)	9.6 km
Tuesday	5.5 km below VT	5.5 km
Wednesday	5.5 km below VT	5.5 km
Thursday	Off	0
Friday	5 * (800m above VT, 800m below VT)	8.0 km
Saturday	8.5 km @ VT	8.5 km
Sunday	7.9 km Fartlek – (2.4 km above VT, 4.5 km @ VT, and 1.0 Km below VT)	7.9 km

7th week – 55.2 km

Monday	3 * (2200m above VT, 1400 m below VT)	10.8 km
Tuesday	7.0 km below VT	7.0 km
Wednesday	6 * (800m above VT, 800m below VT)	9.6 km
Thursday	7.0 km of fun stuff!, below VT	7.0 km
Friday	8.8 km Fartlek – (3.8. km above VT, 4km @ VT, 1km below VT)	8.8 km
Saturday	6.0 km @ VT	6.0 km
Sunday	6.0 km @ VT	6.0 km

8th week - Recovery @ 27.6 km

Monday	7km below VT	7 km
Tuesday	Off	0
Wednesday	2 * (2200m above VT, 1200m below VT)	6.8 km
Thursday	Off	0
Friday	5km @ VT	5 km
Saturday	8.8 Fartlek 3.2 km above VT, 3km @ VT, 2.6 below VT	8.8 km
Sunday	Off	0

Individualized Training Program

Your Training Heart Rates

Training Phase	Heart Rate Range (bpm)	Pulse in 10 secs.
Phase 1 - Below VT	< 150	< 25
Phase 2 - At VT	150 - 165	25 – 28
Phase 3- Above VT	> 165	> 28

bpm – beats per minute

1st Month

Week 1 = 20km
Date: August 16th – 22nd

Day	Workouts	Km	Time
Mon	Off	0	0
Tues	VO_2max test and skinfolds	> 1.6	**Nice Work!**
Wed	Off	0	0
Thur	3 * (1200m above VT, 800m below VT)	6.0	**Done!**
Fri	Off	0	0
Sat	2 * (5 mins. above VT, 5 mins @VT, 5 mins. below VT)	5.2	30 mins
Sun	45 mins @ VT **- OR – Soccer**	7.2	45 mins.

Week 2 = 24.5 km
Date: August 23rd – 29th

Day	Workouts	Km	Time
Wed	Off	0	0
Thur	3 * (8 mins. above VT, 6 mins. below VT)	7.2	42 mins.
Fri	55 mins. below VT - OR - Soccer	7.4	55 mins
Sat	Off	0	0
Sun	2 * (5 mins.above VT, 5 mins.@ VT, 3 mins. below VT)	4.8	26 mins
Mon	30 mins. @ VT	5.1	30 mins
Tues	Off	0	0

Week 3 = 30.0 km
Date: August 30th – September 5th

Day	Workouts	Km	Time
Wed	3 * (9 mins. above VT, 6 mins. below VT)	7.65	45 mins.
Thur	40 mins. below VT -OR - Soccer	5.0	40 mins.
Fri	Off	0	0
Sat	3 * (5 mins. above VT, 5 mins.@ VT, 3 mins below VT)	6.6	39 mins.
Sun	35 mins. below VT	4.5	35 mins.
Mon	40 mins. @ VT	6.3	40 mins.
Tues	Off	0	0

Week 4 = Recovery @ 15 km
Date: September 6th – 12th

Day	Workouts	Km	Time
Wed	Off	0	0
Thur	2 * (9.5 mins. above VT, 6.5 mins. below VT) – OR- Game	6.0	32 mins.
Fri	Off	0	0
Sat	35 mins. below VT -OR - Soccer	4.5	35 mins.
Sun	Off	0	0
Mon	30 mins. @ VT	4.5	30 mins.
Tues	Off	0	0

Week 5 = 36.8 km
Date: September 13th – 19th

Day	Workouts	Km	Time
Wed	3 * (9 mins above VT, 7 mins. below VT)	8.4	48 mins.
Thur	45 mins. @ VT	7.5	45 mins.
Fri	60 mins. below VT -OR Soccer Practise -	8.5	60 mins.
Sat	Off	0	0
Sun	4 * (800m above VT, 800m below VT) – TIMED RUN - OR - 4 * (4 mins above VT, 6 mins below VT)	6.4	40 mins.
Mon	Off	0	0
Tues	35 mins Fartlek -OR Soccer Game -	6.0	35 mins.

Fartlek: A fartlek is a run where YOU decide what you want to do! The idea is that you are changing your intensity throughout the run. For example: You can choose to run hard for a minute and then jog easy for 3 minutes, then a moderate jog for 2 mins....it is completely up to you and depending on how you are feeling. Some examples that you might want to try...

1) Adding in some hills
2) Running backward from time to time – Be careful, it is harder than we think!
3) A real "burner" that we use to do was what we called the "mountain", where we would start at 10 seconds hard, then 10 seconds easy, followed by 20 seconds hard, then 20 seconds easy, and so on and so forth until we reached a minute or a minute and a half...then we would come "back down" the mountain, starting with 1 minutes hard, 1 minute easy, followed by 50 seconds hard, 50 seconds easy, until we reached 0 secs.
4) Pick different landmarks (a tree, a post, a dog, etc...) on your running route and change your speed to that point. For example: Run hard to the big tree, run easy to the next post, and so on.
5) Do some sit-ups and push ups in the middle of the run.
6) Be as creative as you wish!

Week 6 = 45.1 km
Date: September 20th – 26th

Day	Workouts	Km	Time
Wed	50 mins @ VT	8.5	50 mins
Thur	5 * (4 mins above VT, 6 mins below VT)	8.0	50 mins
Fri	40 mins below VT -OR Soccer Practise -	5.5	40 mins
Sat	3 * (9 mins above VT, 9 mins below VT) -OR Soccer Game -	9.6	54 mins
Sun	40 mins below VT	5.5	40 mins
Mon	45 mins Fartlek	7.9	45 mins
Tues	Off	0	0

Week 7 = 55.2 km
Date: September 27th – October 3rd

Day	Workouts	Km	Time
Wed	3 * (10 mins above VT, 10 mins below VT)	10.8	60 mins
Thur	50 mins below VT -OR Soccer Practise -	7.0	50 mins
Fri	35 mins @ VT	6.0	35 mins
Sat	50 mins Fartlek -OR Soccer Game -	8.8	50 mins
Sun	50 mins below VT	7.0	50 mins.
Mon	6 * (4 mins above VT, 6 mins below VT)	9.6	60 mins
Tues	35 mins @ VT	6.0	35 mins

Week 8 = Recovery @ 27.6 km
Date: October 4th – 10th

Day	Workouts	Km	Time
Wed	Off	0	0
Thur	50 mins below VT -OR Soccer Practise -	7 km	50 mins
Fri	2 * (10 mins above VT, 9 mins below VT) -OR Soccer Game -	6.8 km	38 mins
Sat	Off	0	0
Sun	50 mins Fartlek	8.8 km	50 mins
Mon	30 mins @ VT	5 km	30 mins
Tues	YOU ARE DONE!!!	WAY	TO GO!