The effect of the menstrual status on competitive performance and condition

by

Akemi Sawai

A thesis for the Degree for Doctor of Sports Medicine

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Research achievement

【Original paper】

1. **Sawai A**, Tochigi Y, Kavaliova N, Zaboronok A, Warashina Y, Mathis BJ, Mesaki N, Shiraki H, Watanabe, K: MRI reveals menstrually-related muscle edema that negatively affects athletic agility in young women. PloS one, 13(1), 2018 e0191022.
2. **Sawai A** and Bryan J. Mathis, Hiroaki Natsui, Alexander Zaboronok, Risa Mitsuhashi, Yuki Warashina, Noboru Mesaki, Hitoshi Shiraki, Koichi Watanabe: Risk of female athlete triad development in Japanese collegiate athletes is related to sport type and competitive level. International Journal of Women's Health, 10: 671-687, 2018.

【Academic Conference Presentation】

1. **Sawai A**, Tochigi Y, Warashina Y, Shiraki H, Watanabe K: Change of the fluid component in calf and athletic performance in a menstrual cycle. European College of Sport Science, Austria, 2016. 7.
2. **澤井朱美**，栃木悠里子，Nadzeya Kavaliova, Alexander Zaboronok，藁科侑希，目崎登，白木仁，渡部厚一：月経周期に伴う下腿水分量の変動が運動パフォーマンスに及ぼす影響．第72回日本体力医学会大会，愛媛，2017.9.
3. **Sawai A**, Mitsuhashi R, Warashina Y, Zaboronok A, Sone R, Mesaki N, Shiraki H, Watanabe K. Response of Muscle Damage Markers After Acute Heavy Exercise in Different Ovarian Hormone Secretion. American College of Sports Medicine, Minneapolis, 2018. 5.
4. **澤井朱美**，夏井裕明，Alexander Zaboronok，Bryan J. Mathis，藁科侑希，渡部厚一：大学女子アスリートにおけるFemale athlete triad発症リスクと競技強度・競技レベルとの関連性．第21回日本運動疫学会学術総会，東京，2018. 6.

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List of Abbreviation

ACSM: American College of Sports Medicine

AVP: Arginine vasopressin

ANOVA: Analysis of Variance

BMI: Body mass index

BMD: Bone mineral density

CK: Creatine kinase

CLEIA: Chemiluminescence Enzyme Immunoassay

COP: Center-of-pressure

CYC: Cyclic menstruation group

ECN: Executive control network

ER: Estrogen receptor

E2: Estradiol

FAT: Female athlete triad

FP: Follicular phase

FSH: Follicle-stimulating hormone

GnRH: Gonadotropin-releasing hormone

GTG: Group-to-group comparison

HRT: Hormone replacement therapy

LD: Lactate dehydrogenase

LH: Luteinizing hormone

LP: Luteal phase

LTH: Low-to-high competitive comparison

MP: Menstrual phase

MRI: Magnetic resonance imaging

OC: Oral contraceptives

OP: Ovulatory phase

DYS: Menstrual dysfunction

PMS: Premenstrual syndrome

PR: Progesterone receptor

P4: Progesterone

RAAS: Renin-angiotensin-aldosterone system

RIA: Radioimmunoassay

STG: Sport-to-group comparison

Abstract

Eumenorrhea, or normal menstrual periods, is an easily disrupted phenomenon and chronically low levels of ovarian hormones are not uncommon in female athletes. However, the impact of this variation of hormone levels in both regularly and abnormally menstruating athletes and its relation to athletic performance and condition has not been extensively studied. Not only does irregular menstrual function affect performance, but also menstrually related symptoms during regular menstrual cycles may affect competitive fitness. The research presented in this thesis was undertaken to 1) Quantitatively evaluate water retention and its influence on athletic performance during regular menstrual cycles, 2) examine the current state of the female athletic triad (FAT) in Japanese college athletes, 3) quantify muscle damage response due to differing ovarian hormone levels after acute heavy exercise, and 4) examine muscle damage response due to differing ovarian hormone levels after a 4-day training camp.

In the first study, fluid retention in the calves of eumenorrheic female students (non-competitive athletes) was quantitatively evaluated over their menstrual cycle using magnetic resonance imaging (MRI) and the relationship between cyclical water retention and athletic performance were determined. The menstrual cycle was divided into 5 phases: menstrual, follicular, ovulatory, early luteal, and late luteal with sampling done in either morning (AM) or afternoon (PM) sessions. At each phase, MRI of the calf (7:00-8:00, 14:00-16:00), body composition and hormones (7:00-8:00), and athletic performance (14:00-16:00) were evaluated. Estradiol levels decreased significantly in the menstrual phase and the follicular phase compared to the early luteal phase. Menstrual phase estradiol levels were significantly lower compared to the ovulatory phase, and the late luteal phase. Progesterone levels decreased significantly in the menstrual phase and the follicular phase compared to the ovulatory phase, the early luteal phase, and the late luteal phase. AM T2 signals were significantly lower in the menstrual phase compared to the ovulatory phase but no other phases. PM T2 signals increased significantly in the menstrual phase compared to the follicular phase, ovulatory phase, and the late luteal phase, and the

difference between the AM and PM values increased significantly in the menstrual phase compared to the other 4 phases. A negative correlation between fluid retention and agility was observed. Thus, fluid retention fluxing during menses could influence athletic agility.

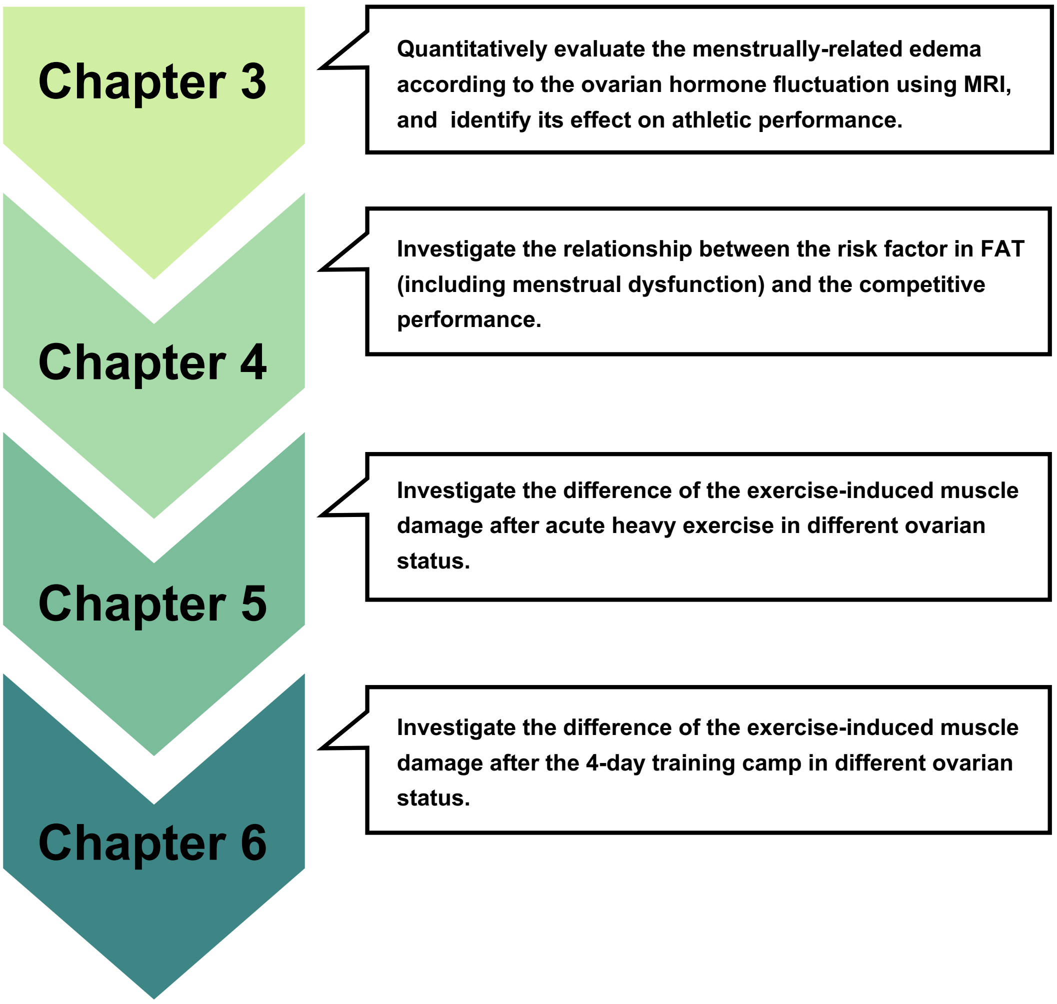
The effect of fluxing on athletic performance was shown to vary over the menstrual cycle correlating with water retention in the low ovarian hormone phase. However, the current state of irregular menstrual status and its effect on competitive performance has not been clearly explained in Japanese athletes. Therefore, the rates of dysmenorrhea, musculoskeletal injuries and poor nutrition in Japanese collegiate athletes were investigated in the second study and the correlation of sport intensity, training volume and competitive level to the risk factors of the female athlete triad (FAT) were examined. Female athletes suffer a higher rate of menstrual problems, muscle and/or skeletal injuries and poor nutrition intake than non-athletic women. Therefore, the link between these problems (known as the FAT and the intensity), training amount and competitive level of college sports in Japanese women were investigated. In this study, 531 Japanese collegiate athletes and 20 non-athletes responded to the Japanese-language questionnaire and a classification system that grouped sports by intensity types was used. Higher sport intensities were found to cause menstrual problems and poor nutrition intake, but higher sports training volume caused more injuries. Competitive level only related to menstrual problems but not as much as to intensity. Therefore, coaches in high intensity or high training volume sports are recommended to take special care (especially with regard to menstrual irregularities, including amenorrhea) to monitor their athletes for FAT risk and maintain competitive performance.

In the third study, the effects of chronically low ovarian hormone levels (as opposed to normally cycling levels) on post-exercise muscle damage biomarkers and tendon stiffness were investigated. Nineteen female college athletes were enrolled into two groups: the menstrual dysfunction group (DYS) and the cyclic menstruation group (CYC). Test conditions (rest or exercise) were taken over a 3-week period. The exercise condition involved 6 sets of 5 squats at 90% 1-RM with a 3 minutes rest between each set while resting consisted of sitting quietly for 30 minutes. Blood chemistry, muscle soreness and tendon stiffness were measured before, immediately after, 30 minutes after, 60 minutes after, and 24 hours after each condition. Menstrual cycles were longer in DYS athletes and estrogen levels were lower in all weeks. CK level in DYS were significantly higher than the CYC group (DYS lowest value: 200U/L pre-exercise vs. CYC highest value: 200U/L 24h after exercise). Biceps femoris tendon stiffness significantly increased 24 hours post-exercise only in the DYS group. To conclude, a chronically low estrogen state due to menstrual irregularity adversely impacts post-exercise soreness, muscle damage and tendon stiffness which suggests that menstrual status must be factored into training and exercise recovery programs.

In the fourth study, the effects of chronically low ovarian hormone levels (as opposed to normally cycling levels) on post-training camp muscle damage biomarkers and tendon stiffness were investigated. Twenty-eight female college basketball players were enrolled into two groups: the menstrual dysfunction group (DYS) and the cyclic menstruation group (CYC) according to their ovarian hormone levels. Ovarian hormone levels, muscle damage markers, blood pressure and heart rates, subjective muscle soreness, scores for emotional distress, and muscle stiffness were evaluated 2 weeks prior to a 4-day training camp (P1), the last day of the training camp (P2), the day after the last day of the training camp (P3), 2 days later (P4), and 2 weeks later (P5).

Estradiol and progesterone levels were significantly lower in DYS compared to CYC. Serum CK and LD levels were elevated in P2 and P3 in both groups with showing a significant difference between groups in P1 and P2. Furthermore, biceps femoris muscle stiffness was significantly elevated in P2 and P3 in both groups while showing a significant difference between DYS and CYC in P3 and P4. Gastrocnemius stiffness was elevated significantly in P3 compared to P1, and only DYS in P4 showed a significant difference between CYC in P2 and P3. Therefore, a chronically low estrogen state due to menstrual irregularity adversely impacts post-training camp muscle damage and tendon stiffness which suggests that menstrual status must be factored into training and exercise recovery program.

The flow of the experimental studies from Chapter 3 to Chapter 6 is shown in Figure 1.



Quantitatively evaluate the menstrually-related edema according to the ovarian hormone fluctuation using MRI, and identify its effect on athletic performance.

Investigate the relationship between the risk factor in FAT (including menstrual dysfunction) and the competitive performance.

Investigate the difference of the exercise-induced muscle damage after acute heavy exercise in different ovarian status.

Investigate the difference of the exercise-induced muscle damage after the 4-day training camp in different ovarian status.

Figure 1. **Flow of the experimental studies**

1. General Introduction

Numbers of female athletes are ever increasing, with the 2016 Olympic Games seeing the highest ever number of women participating (5059 athletes; The International Olympic Committee, 2018). Even within Japan, female athletes who won gold medals outnumbered men in the 2012 and 2016 Olympic games and this trend is expected to increase. Of particular importance to supporting female athletes is the investigation of the unique influence of menorrhea on physical activity and conditioning.

There have been previously reported differences (e.g., physiological, anatomical, psychological) between men and women. The quadriceps angle (Q-angle) is a clinical measure of the alignment of the quadriceps femoris musculature relative to the alignment of the underlying skeletal structures of the pelvis, femur and tibia (Rauh et al., 2007; Livingston, 1998). Women are reported to have a wider Q-angle and considered to have greater risks of anterior cruciate ligament tearing, at about two to eight times greater than men (Wojtys et al., 1998; Gwinn et al., 2000; Alenton-Geli et al., 2010; Sutton et al., 2013). Differences in psychology have been also reported between men and women. Salivary cortisol, a measure of psychological stress, was greater in men than in women after the social stress test (Reschke-Hernández et al., 2017). The rate of infections of pulmonary disease have also been reported to show sex differences. The rate of pulmonary and cardiovascular disease in men were greater than in women (Lozano et al., 2010; Nhamoyebonde and Leslie 2014; Arnold et al., 2017), and this not only because of the differences in smoking rates between the sexes but also the presence of steroid hormones (estrogen, progesterone, and testosterone) has been suggested (Sathish et al., 2015).

Estrogen, Progesterone, and Testosterone are called sex hormones, and are steroid hormones secreted from the ovaries, testes, or adrenal cortex. They affect growth, function of the reproductive organs and the development of secondary sex characteristics. Menstruation (menstrual cycle) is a specific phenomenon in women which is regulated by the sex hormones estrogen and progesterone. These hormones fluctuate rapidly on a 28-day cycle to prepare the uterus lining for pregnancy. It has been discussed previously that the variation of hormone levels may affect athletic performance in athletes (Lebrun 1993 and 1995). Furthermore, these fluctuations of hormone levels lead to the occurrence of menstrually-related symptoms which mainly occur from the premenstrual to menstrual phase. Not only inactive women but also athletes have been reported to suffer with this phenomenon, reporting that the best agility performance was generally recorded in the first postmenstrual days and the worst performance during the premenstrual interval and the first few days of the menstrual flow (Lebrun 1993). Over 80% of Japanese top-level female athletes and over 50% of regular level athletes have been reported to experience menstrual discomfort (Nakamura et al., 2012; Takeda et al., 2015), which raises concerns about their competitive performance. Approximately 10% of athletes are reported to suffer with edema in which estrogen and progesterone are suggested to have direct and indirect influence on bodily fluid deposition in tissues and sodium regulation, thereby influencing menstrual cycle-related edema (Hessemer et al., 1985; Stachenfeld et al., 2004 and 2008). However, the description of the exact mechanisms by which edema and its related symptoms influence female athletic performance is lacking and known mechanisms will be discussed in Chapter 3.

About 59% of the Japanese top-level female athletes have been reported to have a regular menstrual cycle while over 40% of them were reported to have menstrual dysfunction (Nose et al., 2014). Menstrual dysfunction is a common health issue among female athletes and is called the Female Athlete Triad (FAT). Since the American College of Sports Medicine (ACSM) first reported on FAT in 1997 and additionally updated the definition in 2007 (Nattiv et al., 2007), it has been known as a clinical health issue that occurs in female athletes. The FAT consists of three syndromes: low energy availability, menstrual dysfunction, and low bone mineral density. These are strongly interrelated, leading to a restriction in athletes to compete in their best conditions. These three components have been reported to have a strong relationship (Beals and Manore 2002; Torstveit and Sundgot-borgen 2005; Joy and Nattiv 2016) and that one criterion can lead an athlete to two or more of these issues. Several factors are considered to contribute to the development of the triad. Pressure to excel in sports, be thin and/or achieve a low body weight, or insufficient energy availability in general, may lead to disordered eating, and/or menstrual dysfunction, and subsequently loss of bone mineral density potentially resulting in osteopenia or osteoporosis (Otis et al., 1997). Therefore, it should be recognized early so that an intervention can occur before the consequences are too difficult to reverse. However, the risk factors of this phenomenon have not been fully explored in Japanese female college athletes and, additionally, the effect of competitive level on FAT risk factors has also not been reported. Therefore, the FAT risk factors in Japanese female athletes of various sports as well as examine the impact of competitive level on FAT are examined in Chapter 4. As female participation in sports continues to increase and become more competitive, it is important to prevent, diagnose, and manage the components of the female athlete triad in athletes.

Estrogen has been reported to show an important protective role in muscle membrane stability (Tiidus et al., 1999; Tiidus 2000, 2001, and 2003; Carter et al., 2001; Clarkson et al., 2001; Roth et al., 2001). It has been reported previously that estrogen supplementation has been shown to attenuate post-exercise serum creatine kinase (CK) and muscle neutrophil infiltration in animals (Tiidus 2001). Studies in postmenopausal women on hormone replacement therapy (HRT) have shown to have lower serum CK activity following eccentric exercise than those who do not take HRT (Dieli-Conwright et al., 2009). Furthermore, women who were taking oral contraceptives (OC) tested during their active pill phase (and therefore exposed to high exogenous synthetic estrogen) had a lower CK response 72 h after downhill running compared with eumenorrhoeic women not on oral contraceptives when tested in their mid-follicular phase with moderate to low endogenous estrogen concentrations (Carter et al., 2001). However, the physiological response after exercise has been unclear in women (especially in athletes) with ovarian suppressed menstrual status, compared to women (athletes) with regular menstrual function. Therefore, the muscle damage response after acute heavy intensity exercise (Chapter 5) and 4-day training camp (Chapter 6) in athletes with different ovarian secretion status are investigated and examined in those Chapters.

Female athletes need to consider the effect of their menstrual status and ovarian hormone levels on competitive performance and condition. Ovarian hormones are considered to be indicative of women's health.

Therefore, the hypothesis of this study is that athletes with chronic low ovarian hormone levels due to ovarian suppression will experience a negative effect on competitive performance and condition than eumenorrheic athletes. Results from Chapter 3 to Chapter 6 will suggest a better way for female athletes to cope with their menstrual status and give recommendations to athletic trainers, medical doctors, and coaches.

This thesis is presented in 8 main Chapters:

- Chapter 2 presents a review of literature examining the differences between men and women, including hormonal adaption. The menstrual function and cycle in eumenorrheic women and its physiological response in women are discussed. Furthermore, limited literature among low ovarian hormone athletes and its effect to the competitive performance and condition is discussed.
- In Chapter 3, water/fluid retention over the menstrual cycle and its effect on athletic performance is examined.
- In Chapter 4, a survey of irregular menstrual status in collegiate students and its relationship to sports type and competitive level with respect to the female athlete triad is examined.
- In Chapter 5, the response of muscle damage after heavy exercise is investigated in athletes with different menstrual statuses.
- In Chapter 6, the response of muscle damage after a 4-day training camp is investigated in athletes with different menstrual statuses.
- Chapter 7 brings the previous studies together by examining the effect of the variation of hormone levels on athletic performance and condition in both eumenorrheic athletes and athletes with menstrual dysfunction.
- The final Chapter (8) of the thesis summarizes the findings from the experimental work and addresses questions on which future research should focus.

2. Review of Literature

2-1. Introduction

This chapter collates the most pertinent findings from studies on sex hormones (estrogen and progesterone) in women and female athletes. The review of literature has been divided into 5 main sections. Section 2-2 examines the endocrinological differences between men and women. Section 2-3 examines the role of the sex hormones estrogen and progesterone. Section 2-4 reviews the literature on the menstrual cycle and its effect on exercise performance in eumenorrheic, inactive women and athletes, including menstrual disturbances.

2-2. Men and Women

The differences between men and women are pronounced and unavoidable when discussing any aspect of physical performance. In this section, such reported differences in the sports medicine field and the contribution of sex hormones will be examined. During puberty, the physique of boys and girls starts to change, mediated by the sex hormones. Testosterone (the male sex hormone) lowers the voice, broadens the shoulders, and causes the facial structure to become more masculine. In girls, estrogen plays a role in the development of so-called female secondary sex characteristics, such as breasts, wider hips, and axillary hair. Furthermore, most girls experience menarche around this age.

Psychological, anatomical, physiological, and immunological differences have been reported between men and women. The physiological stress response, regulated by the hypothalamic-pituitary-adrenal (HPA) axis, has been reported to be greater in men than in women, assessed by salivary cortisol levels which reflect chronic stress (Kudielka et al., 2005; Zöller et al., 2010). Anatomical sex differences lead female athletes to greater risks of injuries. Anterior cruciate ligament tearing is a common injury in female athletes reported higher frequencies than in males (Ireland 2002) because of a greater Q-angle. Immunological conditions also show sex differences. Females have higher levels of plasma immunoglobulin than men and exhibit more vigorous responses to exogenous antigens, indicating a higher level of humoral immunity in females than in males (Bouman et al., 2004). In the backgrounds of all these reports, the presence of estrogen has been considered to play an important role. According psychologists, large community and clinic-based studies indicate that negative mood complaints (Davydov et al., 2005; Gonda et al., 2008) and suicidal behavior (Baca-Garcia et al., 2004; Saunders et al., 2006) increase in women, even in healthy women. It has been reported previously that brain areas which are central to mood regulation show some of the largest densities of estrogen receptors in the human brain (Ostlund et al., 2003; Merchenthaler et al., 2004) and that estrogen may modulate the activity of these areas. Estrogen has been also reported to affect the flexibility and stiffness of ligaments (especially in anterior cruciate ligament) leading to stiffer ligaments in men than in women (Hsu et al., 2006; Boguszewski et al., 2015). Estrogen receptors are known to be present in fibroblasts of tendon and ligaments (Kjaer et al., 2008) and are thought to be responsible for this difference. Estrogen has been reported to affect immune function along with progesterone (Paavonen 1994). Women have been reported to have fewer blood monocytes and NK cells but more CD4+ cells and more neutrophils than men (Willemsen et al., 2002; Bouman et al., 2004) and women appear to suffer from fewer viral infections (Beery 2003). According to these previous reports, the presence of sex hormones should be an important consideration in sports performance.

2-3. Presence of sex hormones

Estrogen, progesterone and testosterone are all derived from cholesterol via biosynthesis pathways (Payne and Hales, 2004; Ghayee and Auchus, 2007) called steroidogenesis (Figure 2.1). Cholesterol is converted to pregnenolone by a P450 side chain cleavage (P450scc) enzyme located in the mitochondrial membrane and then the specific hormone produced depends on the tissue and enzymes present (Miller et al., 2011). Pregnenolone is then converted to either progesterone (via 3β-hydroxysteroid dehydrogenase) or dehydroepiandrosterone (DHEA via cytochrome P450 17) both of which may be further converted into androstenedione (via cytochrome P450 17 or 3β-HSD, respectively).

Estrogen is produced from the conversion of testosterone into estrone or from the conversion of androstenedione into estradiol via the aromatase enzyme while estriol is converted from estrone and estradiol via the liver and placenta. Estradiol (E2) can be also produced from the conversion of estrone via 17βhydroxysteroid dehydrogenase (Gardner 2011).

Hormone production is regulated by the neuro-endocrine axis throughout life. Gonadotropin-releasing hormone (GnRH) (Gardner 2011), which is released from the hypothalamus, stimulates the anterior pituitary gland to release luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Gardner 2011). These hormones regulate the production of steroidogenesis in the ovaries in a feedback-dependent manner to regulate the secretion of gonadotropin and ovarian function. Estrogen is produced in the granulosa cells of the developing follicle (during the proliferative phase) and the corpus luteum, while progesterone is produced in the corpus luteum during the luteal phase in the menstrual cycle.

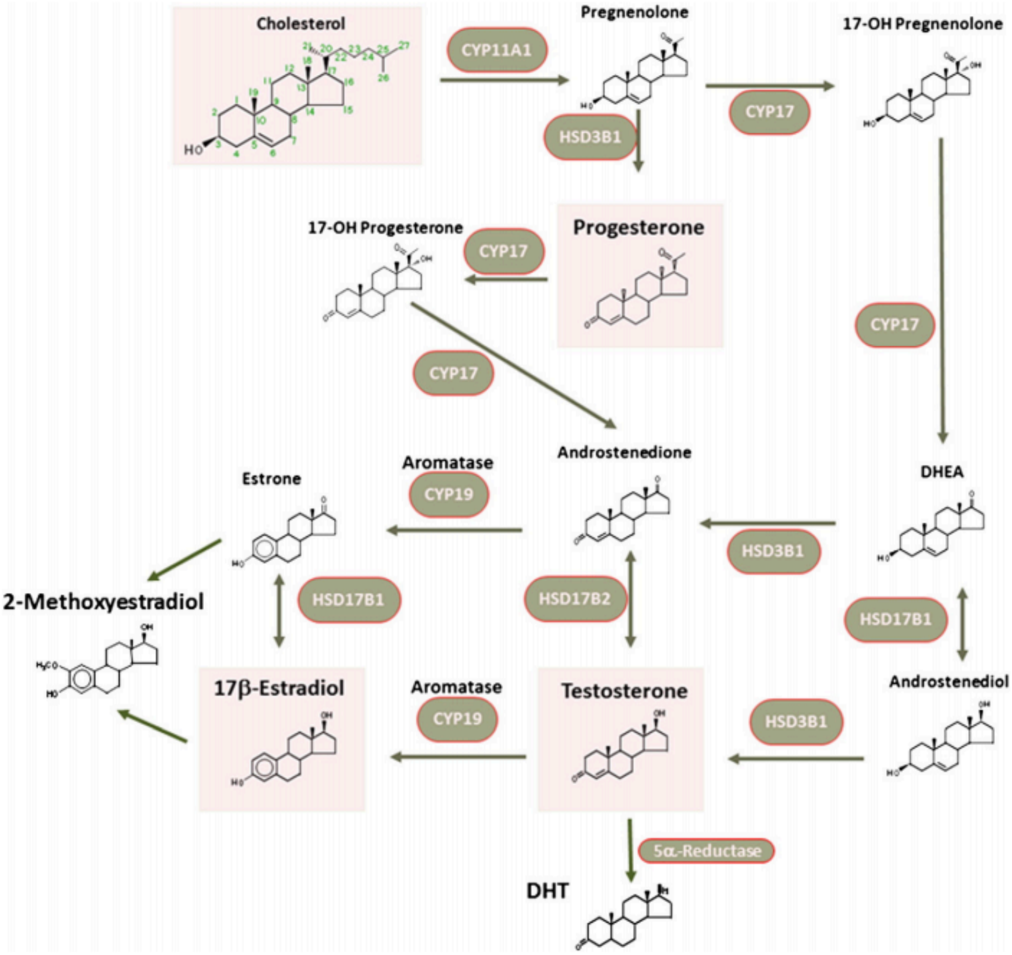


Figure 2.1 Synthesis of estrogen, progesterone, and testosterone from cholesterol. (Sathish et al., 2015)

2-3-1. Estrogen

In both males and females, production and conversion of estrogen occurs in the brain, adipose tissue, muscle, and skin (Gardner 2011). Approximately 80% of estrogen is produced by the peripheral glands in men while the rest is by the Leydig cells in the testes (Gardner 2011). In women, 95% of estrogen production occurs in the granulosa cells of the developing follicle during the proliferative phase and the corpus luteum during the luteal phase of the menstrual cycle (Gardner 2011). Estradiol (E2) is the major form of circulating estrogen in non-pregnant females of reproductive age while estrone is the dominant type of estrogen present in postmenopausal women and has one third of the potency. Estriol is biologically relevant only during pregnancy (Gardner 2011).

Estrogen effects are commonly mediated by two estrogen receptors, ERα and ERβ, acting via both classical, genomic mechanisms of altered protein expression/function, as well as acute, non-genomic mechanisms that are being increasingly recognized. In addition to these classical receptors, recent studies have shown that GPR30, a member of the G protein-coupled receptor superfamily, mediates estrogen-dependent kinase functional activation and transcriptional responses (Filardo et al., 2002; Prossnitz et al., 2008). Accordingly, cellular effects of estrogen are likely dependent on the relative expression and actions of ERα vs. ERβ, vs. GPR30, which makes for complex signaling within any tissue. Classical ERα and ERβ both belong to the nuclear receptor family of transcription factors (Heldring et al., 2007; Miller & Duckles, 2008) and are encoded on different genes, with conserved homology in the ligand-binding domain and DNA binding regions, but significant variability at the N-terminus (Heldring et al., 2007). Although ERα and ERβ have similar affinities towards 17-β-estradiol (E2), several differences in transcriptional activation levels have been observed. Some studies suggest that, when both ERs are expressed in a cell, ERβ inhibits ERα-mediated transcription (Liu et al., 2002; Lindberg et al., 2003). For example, activation of ERα can lead to aberrant proliferation (Teng et al., 2008), whereas ERβ activation has antiproliferative effects via counteraction of ERα effects (Lahm et al., 2008).

2-3-2. Progesterone

Progesterone (P4), the second major endogenous female steroid hormone, is primarily produced by steroidogenesis in the corpus luteum during the LP of the menstrual phase (Gardner 2011). Granulosa cells convert thecal androgens to progesterone in this phase while Leydig cells produce progesterone in males (Gardner 2011). P4 is involved in variety of physiological and metabolic changes throughout life including embryogenesis, puberty, menstrual cycle, and pregnancy. P4 is mainly produced in the ovaries, although small amounts are produced in the brain and adipose tissue as well. P4 exerts its effect via progesterone receptors (PRs) with target gene transcription. Mammals have two types of PRs: PR-A and PR-B and both these isoforms are transcribed from same gene, although PR-A differs in the truncated N-terminal domain (Edwards, 2005). Both PRs, PR-A and PR-B has similar ligand binding affinities, but their transcriptional activation differs. For example, PR-B is a strong promoter of transcriptional activities in multiple cell types whereas PR-A isoform is not (Giangrande & McDonnell, 1999). When activated, PRs can recruit regulatory proteins such as SRC-1, SRC-2, SRC-3, CBP/p300 (McKenna et al., 1999) and may also modulate histone acetylation/deacetylation and chromatin remodeling. In addition to this nuclear receptor function, PRs are able to promote and regulate multiple cellular signaling mechanisms independent of nuclear activation (Gellersen et al., 2008). Furthermore, P4 also has been reported to show an antiestrogenic physiological effects (McMurry et al., 2000).

2-4. Menstrual cycle

After puberty and before menopause, females experience cyclic changes in estrogen and progesterone within their menstrual cycle. The hypothalamic-pituitary-ovarian axis regulates the menstrual cycle via a feedback system (Bruce et al., 2009; Deveto et al., 2012). The hypothalamus secretes GnRH which acts on the pituitary gland in a pulsatile manner and stimulates the secretion of the gonadotropins follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) (Bruce et al., 2009; Deveto et al., 2012). It is the frequency and amplitude of these pulses which determine the quantity of each hormone ultimately secreted. Slower frequencies appear to precipitate FSH secretion, whereas LH secretion has a predilection for higher frequencies of GnRH stimulation (Deveto et al., 2012).

At the start of the menstrual cycle, the ovary contains several antral follicles, each consisting of an oocyte, that are separated from the antrum, both of which are surrounded by a layer of granulosa cells (cumulus cells and mural cells respectively). These cells are surrounded by a basal membrane, around which lies another layer of theca cell. Theca cells develop LH receptors if they are part of the dominant follicle and produce androgens (P4 or testosterone) from cholesterol. Conversely, granulosa cells have FSH receptors; androgens are absorbed by these cells and metabolized to E2 (Bruce et al., 2009; Deveto et al., 2012). Granulosa cells also produce the peptide hormone inhibin, which includes two isoforms, A and B (Bruce et al., 2009; Deveto et al., 2012). In the late luteal phase (prior to menstruation) and the early follicular phase, levels of circulating FSH rise. This in turn stimulates follicular development and leads to selection of a dominant follicle. Whilst it is not known exactly how a dominant follicle is selected, it is thought that through varying follicular sensitivity, the most sensitive follicle goes on to mature, whilst the other follicles undergo atresia (degeneration). With its development, the dominant follicle secretes increasing levels of E2; this acts on the endometrium to stimulate proliferation. At the pituitary gland, rising levels of E2 and inhibin B act to reduce FSH secretion through a negative feedback mechanism (Bruce et al., 2009; Deveto et al., 2012).

During the early and mid-follicular phases, E2 also exerts negative feedback on LH secretion, which ensures basal levels during this period. However, about 36 hours prior to ovulation (i.e. in the late follicular phase), E2 reaches levels in the circulation which switch this negative feedback effect to a positive feedback effect. This leads to a surge in LH (which is accompanied by a smaller surge in FSH) over a 24-hour period in the 24 hours prior to ovulation. This LH surge leads to rupture of the dominant follicular wall and release of the oocyte (Deveto et al., 2012).

Following ovulation, there is an abrupt fall in E2 production from the ruptured follicle. The follicle undergoes a series of changes which convert it into an endocrine structure called the corpus luteum ("yellow body"). This produces E2 and progesterone which act on the endometrium to promote implantation. LH maintains the corpus luteum in the week following ovulation, but if pregnancy does not occur, then this begins to degenerate, leading to a gradual reduction in the production of steroid hormones. With falling E2 and progesterone levels, the loss of negative feedback leads to a subsequent rise in FSH, heralding the start of a new menstrual cycle. A summary of these processes is shown in Figure 2.2 (Bruce et al., 2009; Deveto et al., 2012).

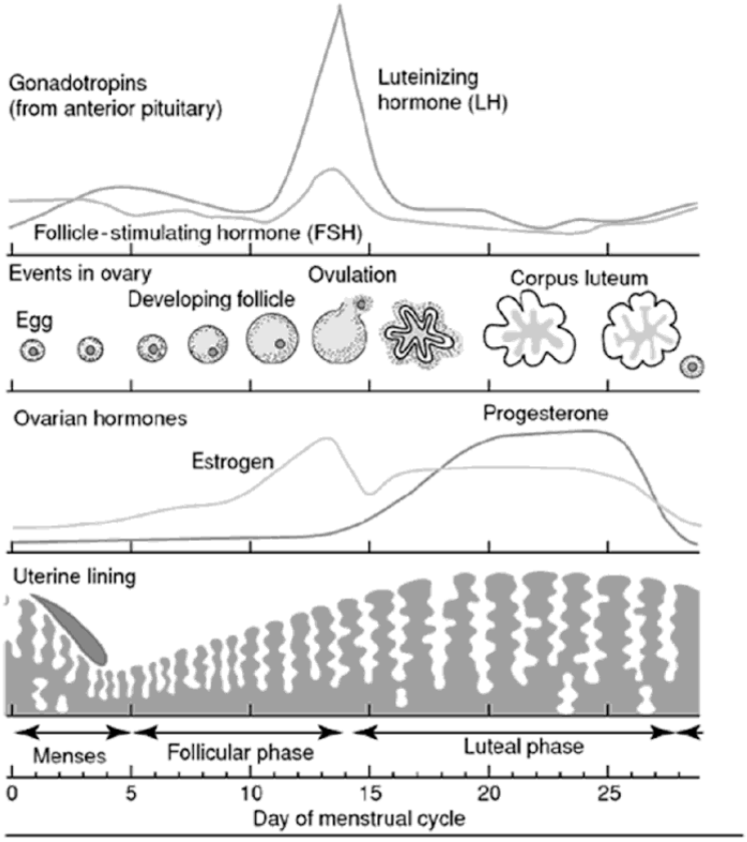


Figure 2.2 Diagram of hormonal fluctuations in the menstrual cycle

(Bruce et al., 2009; Deveto et al., 2012)

2-4-1. Menstrually related reports in inactive women

Menstruation is a specific phenomenon in women which lasts for approximately 40 years. The menstrual cycle in women is characterized by high variability in cycle length (26-38 days), 5-days of menses, a fertile phase from 5 days before to the day of ovulation, and low fertility which is dependent on cycle length and age (Harlow 2000). It has been reported that over 90% of female collegiate students experience menstrual discomfort during their menstruation (Noda 2003). Symptoms occurring during the premenstrual to menstrual phase are called “menstruation-related illness” since Frank et al. (1931) had first reported this illness as “premenstrual tension” and this has been reported to influence quality of life (QOL) in Japanese women (Kaimura et al., 2014). The variation of the ovarian hormones has also been reported to alter cognitive function. A high connectivity between the hippocampus and the bilateral superior has been reported in the parietal lobe in the late follicular phase, which is a high estrogen-only phase that only estrogen in normal healthy women (Lisofsky et al., 2015). Additionally, women in follicular phase showed increased connectivity with the executive control network (ECN) relative to women in luteal phase in the right anterior cingulate cortex (Petersen et al., 2014). As it has been estimated that women experience menstruation more than 400 times over a normal lifespan, this should be considered to improve their QOL. Throughout the pre-ovulation or follicular phase (FP), plasma E2 and P4 are at low concentrations while during the post-ovulation or luteal phase (LP) both are high. Both E2 and P4 can affect energy substrate metabolism (Hackney 1999), thermoregulation (Kolka and Stephenson 1997) and body water and electrolyte homeostasis (De Souza et al. 1989), all of which are crucial to exercise status. Therefore, the different hormone environments in the FP and LP of the menstrual cycle have the potential to impact both exercise capacity and exercise performance. Additionally, menopause is a normal part of aging when it happens after the age of 50 and is characterized by low ovarian levels in women. Estrogen is a crucial hormone for osteoclast inhibition and for preventing osteoporosis (Wu et al., 2015); however, due to the characteristics of menopause, bone density decreases in women with menopause. Menstrual cycling and its related symptoms affect inactive women in various ways according to the fluctuating levels of estradiol and progesterone.

2-4-2. The effect of the menstrual cycle on eumenorrheic female athletic performance and condition

Increased participation in sport by women has led to enhanced interest in the physiological and metabolic responses of women to sport and exercise (Constantini et al., 2005). Estrogen has been reported to show a metabolic action (Bunt 1990; Oosthuyse et al., 2010), alter and regulate the cardiovascular system (Sarrel 1990; Collins et al., 1993; Collins 1996a; Collins 1996b; Collins et al., 1996), regulate electrolyte metabolism, bone metabolism and neuroendocrinological function while progesterone modulates body temperature (Hessemer et al., 1985), minute ventilation (Schoene et al., 1981; Dombovy et al., 1987), carbohydrate and lipid metabolism (Reinke et al., 1972), and water retention (Olson et al., 1996). Women exhibit large and predictable hormonal variations across the menstrual cycle (Speroff and Fritz 2005) and therefore, must be considered to have the possibility of altering competitive performance and condition (Table 2.1).

	First Author, Date	Type of population	Methods of measurement	Comparison of phases in MC	Findings
Muscle strength	Pöllänen et al, (2015)	Premenopausal, Postmenopausal HRT user Postmenopausal non-HRT user	Knee extension strength (N) , Vertical jump height (cm), Thigh CSA (cm^2)	1-5 days of estrous cycle in premenopausal women	Thigh CSA: Premenopausal > Postmenopausal and Post HRT > Post non-HRT
	Sarwar et al, (1996)	Sedentary women (OC user and non-OC uer)	Quadriceps strength(N), Handgrip strength (N), Fatiguability (FI)	Early Follicular (EF) Mid-Follicular (MF) Mid-Cycle (MC) Mid-Luteal (ML) Late-Luteal (LL)	Quadriceps and Handgrip strength: MC > EF, MF, ML, LL
	Phillips et al, (1996)	Athlete (rowers) Sedentary women (OC, non-OC) Sedentary men	Adductor pollicis muscle strength (N), Adductor pollicis CSA (mm^2)	Follicular phase (FP) Ovulatory phase (OP) Luteal phase (LP)	Adductor muscle strength: FP > OP, LP
	DiBrezzo et al, (1991)	Inactive women with regular menstrual cycle (MC)	Knee flexors and extensors muscle strength (N)	Mensus Ovulation Luteal	No difference
	Lebrun et al, (1995)	Athletes with regular MC	Knee flexors and extensors muscle strength (N)	Follicular phase (FP) Luteal phase (LP)	No difference
	Gür et al, (1997)	Sedentary women	Knee flexors and extensors muscle strength (N)	menstrual phase Follicular phase Luteal phase	No difference
Endurance	Schoene et al, (1981)	Inactive women, athletes with regular MC Athletes with amenorrhea	Resting and exercise voluntary exhaustion (VE) Exhalation gas analysis	Follicular phase Luteal phase	Resting VE: FP > LP in inactive women and athletes with regular MC
	Smekal et al, (2007)	Athletes with regular MC	Exercise VE Exhalation gas analysiss	Folicular phse Luteal phase	No difference
	Vaiksaar et al, (2011)	Athletes in competitive level (non-OC) Recreationally trained athletes (OC and non-OC user)	VE during rowing ergometer	Folicular phse Luteal phase	No difference in all group
	O' Leary et al, (2013)	Athletes with regular MC	70％VO$_2$max and respiratory exchange ratio (RER) during 60minutes treadmilll running	Mid-Follicular phase Mid-Luteal phase	No difference
	Wiecek et al, (2016)	Inactive women	VO2max, Pomax, HRmax	Folicular phse Luteal phase	No difference
Sprint	Julian et al, (2017)	Female soccer players	3 × 30m sprint (sec)	Follicular phase Luteal phase	No difference
	Tounsi et al, (2017)	Female soccer players	Repeated shuttle-sprint (sec)	Early-Follicular phase Late-Follicular phase Luteal phase	No difference
	Pestana et al, (2017)	Normal women with regular MC	Maximal power (W), Heart rate (bpm), and Fatiguability (%) during Wingate test	Mid-Follicular phase Late-luteal phase	No difference
	Tsampoukos et al, (2010)	Athletes who are envolved in sprint or power events	Power (P), Fatiguability (%), and speed (s) in the repeated 30-s sprint	Follicular phase Ovulatory phase Luteal phase	No difference
	Sunderland et al, (2011)	Athlete who are envolved in sprint events (OC, and non-OC user)	Power (MPO, PPO) in the 30-s sprint test	Mid-follicular phase Mid-Luteal phase	No difference
	Middleton et al, (2006)	Female collegiate students who have daily moderate physical activity	Work (W), Power (P), and Fatiguability (DO) in 6-s cycle ergometer × 10 set	Follicular phase Luteal phase	Mean work (MW): Luteal phase > Follicular phase
Agility	Hashimoto et al, (2001)	Collegiate handball players	side-step (point) 25-m shuttle run (sec)	Mensrual phase Follicular phase Luteal phase	25-m shuttle run (sec) : Menstrual phase > Follicular phase
	Sawai et al, (2018)	Inactive women	Side-step (point)	Menstual phase (MP) Follicular phase (FP) Ovulatory phase (OP) Early-luteal phase (ELP) Late-luteal phase (LLP)	Side-step (point) : FP, OP > MP
Balance ability	Aihara et al, (2017)	Inactive women	Dynamic balance ability using Star Excursion Balance Test (SEBT)	Menstrual phase Follicular phase	SEBT score (forward) : Follicular phase > Menstrual phase
	Sawai et al, (2018)	Inactive women	Static balance ability using force plate	Menstual phase (MP) Follicular phase (FP) Ovulatory phase (OP) Early-luteal phase (ELP) Late-luteal phase (LLP)	Total locus length : Menstrual phase > Ovulatory phase
	Hayashi et al, (2004)	Youn women who have daily physcial activity	Dynamic balance ability Static balance ability	Menstual phase (MP) Follicular phase (FP) Ovulatory phase (OP) Early-luteal phase (ELP) Late-luteal phase (LLP)	Locus length per area (cm / sec) : LLP > FP, OP, ELP
Jump ability	Giacomoni et al, (2000)	Athletes with reglar MC (OC and non-OC user)	Maximal cycling power (Pmax$_c$), maximal jumping power (Pmax$_j$), or maximal height of jump (h$_j$) in force-velocity, multi-jump, and squatting jump tests	Menstruation Mid-Follicular phase Mid-Luteal phase	Mid-FP > Menstruation, Mid-LP

Table 2.1 The effects on the athletic performance in eumenorrheic women

Yo-Yo intermittent recovery test scores, a reliable measure for endurance and stamina levels, were reported to be low in mid-LP (characterized as high estrogen and progesterone level) compared to early FP (characterized as low estrogen and low progesterone level) in elite female soccer players (Julian et al., 2017). However, in rowers, there were no significant differences between FP and LP using a stepwise, incremental rowing ergometer test (Vaiksaar et al., 2011).

In sprinting ability, better performance during FP has been shown for a single swimming sprint and for repeated sprint cycling (Bale et al., 1985; Parish et al., 1987), whereas other reports showed better performance during the LP for a single cycle sprint and repeated cycling sprints (Masterson 1999; Middleton et al., 2006) and several reports showed no differences over the menstrual cycle (Tsampoukos et al., 2010; Wiecek et al., 2016). Furthermore, static balance ability (Hayashi et al., 2004), jumping ability (Giacomoni et al., 2000), and agility (Hashimoto t al., 2001) were reported to show better performance in phase with high estrogen levels.

Menstrual cycling according to the variation of hormone levels has been reported to affect athletic physical conditioning. The worldwide known number of women suffering from premenstrual syndrome (PMS) differs by country and study, with 41% of athletes and 59% of non-athletes among Iranian students (Rezaeian 2015), in Turkey, 2 studies found 55.88% and 66.11% (Fekr et al., 2012), and 37.76% and 46.89% (Tulin 2012), respectively, and in 42.4% of female athletes in Poland (Czajkowska et al., 2015). A survey of menstrually related symptoms among Japanese top- level athletes reported (Japan Institute of Sports Sciences 2013) low back pain, decreases in psychological condition, and edema in 10% of athletes.

Considering the effect on competitive performance, menstrually-related pains are reported to effect jump ability and agility (Giacomoni et al., 2000; Hashimoto et al., 2001). However, the occurrence of water retention has been reported to distress athletes not only in the premenstrual phase but also during menstruation (Japan Institute of Sports Sciences 2013). Water retention related to the variance of ovarian hormone levels has not been investigated and the effect on athletic performance needs to be discussed.

2-4-3. Menstrual disturbances with low ovarian hormone levels in athletes

Abnormalities of the reproductive system are encountered in 6 to 79% of females involved in sports activities (Warren et al., 2001). Furthermore, female athletes involved in a wide variety of sports, including runners, swimmers, tennis players, ballet dancers and gymnasts, present with delayed menarche (Warren 1980; Peltenburg et al., 1984; Marcus et al.,1985; Baxter-Jones et al., 1994; Erladson et al., 2008). Menstruation represents a particularly delicate function and its regularity reflects normal reproductive activity. Regular interplay between the hypothalamus, pituitary, ovaries and endometrium give rise to predictable cyclic menses that signify regular ovulation. Ovarian function and menstrual regularity depend on normal cyclic pituitary gonadotropin stimulation. The secretion of gonadotropins occurs in response to pulsatile GnRH release from the hypothalamus which is regulated by various neurotransmitters and neuropeptides. Female athletic performance has been associated with a broad spectrum of menstrual dysfunction, ranging from primary amenorrhea or delayed menarche to luteal phase deficiency, oligomenorrhea, anovulation and secondary amenorrhea. The term amenorrhea refers to the absence of menses. The reproductive dysfunction in amenorrhea is characterized by infrequent or absent LH pulses accompanied by suppressed follicular development, ovulation and luteal activity, leading to persistently low levels of estrogens and P4 and absence of endometrial proliferation (Loucks et al., 1989). Primary amenorrhea is diagnosed when there has been a failure to menstruate by the age of 15 years in the presence of normal secondary sexual characteristics or within five years after breast development if this occurs before the age of 10 (Redman et al., 2005).

Secondary amenorrhea is defined as the absence of three or more consecutive menstrual cycles following menarche, while oligomenorrhea describes menstrual cycles longer than 35 days or menstrual intervals of 45 to 90 days. Luteal phase deficiency denotes asymptomatic subclinical menstrual disturbances resulting in low estradiol levels in the early follicular phase and decreased but normal LH pulse frequency with increased pulse amplitude. Although ovulation occurs, the developed corpus luteum produces reduced progesterone support for adequate endometrial development in the secretory phase. Thus, successful implantation of the fertilized egg is prevented, and infertility ensues. Anovulation is a more severe asymptomatic reproductive dysfunction characterized by suppressed follicular maturation leading to lack of ovulation. Both estrogen and

P4 levels are low, but some proliferation of endometrium is achieved resulting in profuse bleeding at irregular intervals. The benefit of having regular menstrual cycle in female athletes has been discussed for a long time according its negative physical / psychological effect which occurs cyclically. In Japan, approximately 40 % of high-level athletes have been reported to have menstrual dysfunction, mostly by primary amenorrhea, secondary amenorrhea, polymenorrhea, or irregular cycling (Nose et al., 2014). Low ovarian hormone disorders, especially in athletes suffering from chronic amenorrhea, have been reported to cause a higher risk of injuries (especially bone stress fracture) (Nose et al., 2014), higher negative scores in psychological evaluations (Cockerill et al., 1992), low levels of allopregnanolone (an endocrine hormone reported to suppress depression) and high levels of cortisol and ACTH (Meczekalski et al., 2000), low levels of salivary IgA secretion and higher instances of upper respiratory tract infection symptoms than eumenorrheic athletes (Shimizu et al., 2012). Previously, the International Olympic Committee introduced a more comprehensive, broader term for these overall symptoms related to the FAT called "Relative Energy Deficiency in Sports" (RED-S) and suggested health consequences, including menstrual dysfunction, leading to negative effects on athletic performance (Mountjoy et al., 2014; De Souza et al., 2014). Accordingly, the American College of Sports Medicine suggests that emphasis should be placed on optimizing energy availability for prevention by increasing body weight and body fat, HRT, and reducing training volume (Stand position 2007). However, in terms of realistic goals, it is a difficult and unacceptable issue for athletes to reduce their training volume and gain weight for even a short term over a year, especially in aesthetic athletes. Furthermore, medical treatments, such as hormone replacement therapy and oral contraception, will be a major burden for athletes in financial and time restriction and is impractical for prolonged use. Therefore, it is necessary for trainers and coaches to also understand the influence of menstrual status on physical condition.

Low levels of estrogen and P4, seen in athletes with menstrual disturbances (including amenorrhea), negatively influence athlete. Athletes with amenorrhea have shown significantly low saliva IgA secretion rates compared to eumenorrheic athletes (Shimizu et al., 2012), which is suggested to correlate with the serum E2 level (Van et al., 2010). However, low estrogen and progesterone levels have not been investigated as to their effect on competitive performance and/or physiological condition after exercise.

2-5. Summary

It is important to consider each athlete's individual menstrual phase and condition with regard to the variation of ovarian hormone levels. As for athletes with prolonged low ovarian levels, the American College of Sports Medicine (Stand position 2007) suggests that emphasis should be placed on optimizing energy availability for prevention by increasing body weight and body fat, reducing training volume, and HRT. However, changing an ingrained mentality and altering regimens such as diet and exercise can be quite difficult for athletes, especially in endurance, aesthetic, and weight-class sports which have been reported to have a greater risk of developing the FAT (Nose et al., 2014). Prescription of oral contraception and hormone replacement therapy have been also suggested as a solution to this issue. Taking oral contraception may avoid menstrually-related symptoms but only 1-2% of Japanese top-level athletes have been reported to take OC (Nose et al., 2014), while 83% of European female athletes usually take OC for these reasons (Rechichi et al., 2009). Rates of OC usage are highest in young adults: 27% of women aged 20 to 24 use OCs (Jones et al., 2012). Moreover, 85% of women in the United States will use an OC for about 5 years at some point in their lifetime (Chadwick et al., 2012). Therefore, consideration in Japanese athletes with low ovarian hormone levels (even if it lasts for only some months) should be important and investigated to treat athletes in more effective way along with non-pharmaceutical interventions such as meal improvement.

3. MRI reveals menstrually-related muscle edema that negatively affects athletic agility in young women

3-1. Introduction

Currently, the number of female athletes is increasing, with the 2016 Olympic Games seeing the highest ever number of female athletes participating (The International Olympic Committee 2016). Of particular importance to supporting female athletes is the investigation of the unique influence of menorrhea on physical activity and conditioning. About 80% of Japanese top-level female athletes and over 50% of regular female athletes in Japan experience menstrual discomfort (Nakamura 2012; Takeda et al., 2015) and 10% are afflicted by edema (Japan Institute of Sports Sciences 2013), which are thought to be somatosensory factors that influence conditioning by increasing fear and anxiety during training.

In previous reports, estrogen and progesterone were suggested to have direct and indirect influence on bodily fluid deposition in tissues and sodium regulation and thereby influence menstrual cycle-related edema (Stachenfeld et al., 2004 and 2008). These hormones are reported to influence exercise (Bonen et al., 1979; Jurkowski et al., 1978) and athletic performance is affected by symptoms occurring before menstruation which are collectively called premenstrual syndrome (PMS) (Lebrun 1993; Bale et al., 1985). However, the description of the exact mechanisms by which edema and its related symptoms influence female athletic performance is lacking (Olson et al., 1996).

The aim of this Chapter is to quantitatively assess fluid retention associated with the menstrual cycle in healthy young women using MRI. Such quantitative evaluation of edema associated with the menstrual cycle could be important to understand changes in physical condition and help to identify both the reasons for variation of water balance in the lower limbs and its effect on athletic performance.

3-2. Materials and Methods

3-2-1. Study population

Initially, data for 13 female undergraduate and graduate students (23.5±0.4 years old, 160.4±1.4 cm height) of the University of Tsukuba were analyzed. The participants were recruited from May 1st to June 30th, 2015. The inclusion criteria were as follows: age 20 to 25 years, menarche occurred at least 5 years before the start of the study, regular menstrual cycle, normal physical activity level (no professional athletes), physically healthy without any serious illnesses, and non-smokers, and not taking medications (including oral contraception). All participants received an explanation of the purpose and the flow of the study and signed an informed consent form prior to their inclusion. The noninvasiveness of the imaging ensured the safety of the participants. All aspects of the study were approved by the Ethics Committee of the University of Tsukuba (#27-23).

3-2-2. Study procedures

Starting at least 2 months prior to the MRI measurements for edema, the participants measured basal body temperature every morning after awakening and recorded it in a graphical format. Normal menstrual cycles and ovulation were confirmed by a gynecologist based on basal body temperature data analysis and luteinizing hormone surge was detected using a specific urine test (DotestLHa, Rohto Co. Ltd, Osaka, Japan). All subjects maintained the body temperature measurements until the end of the study to confirm the stability of the cycle.

The menstrual cycle was divided into 5 phases: the menstrual phase, day 1 to 4 (menses); the follicular phase, day 7 to 10; the ovulation (luteinizing hormone surge±1 day); the early luteal phase (within 7 days of the post-luteinizing hormone surge); and the late luteal phase (after the early luteal phase until menses). The measurements were carried out on one day between the first and the last days of each phase, excluding the first day of the menstrual phase.

In healthy individuals, no exact MRI signal intensities have been defined for the description of menstrually-related edema, which is usually related to the extension of the interstitial space, deposition of intracellular or extracellular water in various pathologies and is characterized by the increase of T2-signal intensity (May et al., 2000; Sica 2003). Skeletal muscle edema has been evaluated by limb circumferences

(Thompson et al., 1997), limb volume (Paddon-Jones and Quigley, 1997), computer modeling (Bednarczyk et al., 1992), and the spin-spin relaxation time from T2-weighted magnetic resonance skeletal muscle images (Babul et al., 2003). Magnetic resonance imaging (MRI) is increasingly used to study the shift of ions and water from intracellular to extracellular muscle compartments (Nosaka and Clarkson et al., 1996; Nosaka and Newton 2002). The T2 relaxation decay of tissue water is most conveniently obtained from a multi-echo MR sequence in which the signal is refocused at multiple times during one acquisition. MRI signals primarily originate from hydrogen protons found in water molecules, which have high mobility, leading them to possess moderate to long T2times (>10 ms). Edema *per se* is the result of unbalanced distribution of water, manifested as T2WI signal abnormalities on MRI (Y Xiong et al., 2014). T2-weighted proton images have been particularly useful in detecting differences in the chemical environment of cellular water, although T1 relaxation has also been investigated. An increase in extracellular space causes a prolongation of the T2 relaxation time due to increased mobility of water protons compared to the intracellular space (Does and Snyder, 1996). Therefore, to identify the relationship between stages of the menstrual cycle and water deposition in the lower limbs, the intensity of the T2 signals in the calf was compared twice a day over the 5 phases of the menstrual cycle with the parallel measurement of the calf circumference.

In the morning (7:00-8:00 AM) analysis of body composition, blood samples for hormone analysis, measurement of the T2 signal intensity in the calf, and calf circumference were done. In the afternoon (2:00-4:00 PM), the measurement of the T2 signal intensity in the calf, the calf circumference, and tested athletic performance (static balance, vertical jumping ability, agility, and muscle strength in ankle isometric flexion and dorsal flexion [Figure 3.1]) were done. One week before initial tests and during the whole study the participants were asked to have meals on a regular basis with normal water consumption (without excessive hydration), without any alcohol, caffeine, or high-intensity physical exercises. The measurements of each of the participants' parameters began independently with each individual's menstrual cycle phase in random order—the first phase of the measurement of each participant being randomized to prevent habituation effect.

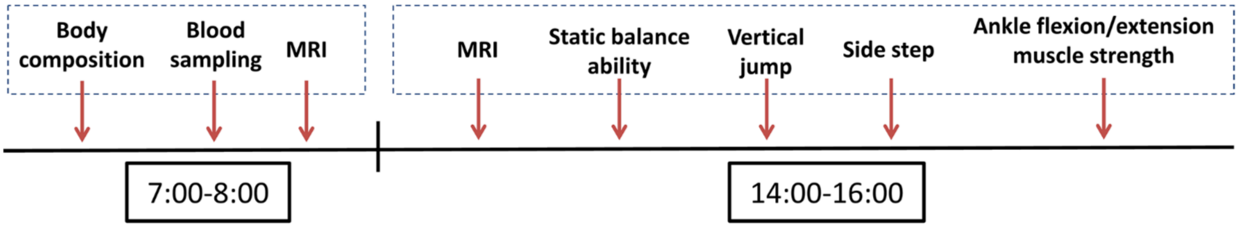


Figure 3.1 Flow of the measurements in each phase of the day.

3-2-3. Measurements

The basal body temperature was measured orally using digital thermometers (CTEB503L, CITIZEN Co, Ltd., Tokyo, Japan). A body composition analyzer (Inody770, Inbody Japan, Inc., Tokyo, Japan) were used to obtain the anthropometric data on 30 indexes, including height, weight, body fat mass and volume, body water volume, lean body mass, and body mass index (BMI). Serum estradiol and serum progesterone concentrations were measured using Chemiluminescence Enzyme Immunoassay (CLEIA) and serum aldosterone concentration was measured using Radioimmunoassay (RIA).

The maximum calf circumference out of 3 measurements in the dominant leg was recorded. The position of the measurement was decided on the first day and marked at a certain distance from the popliteal fossa for each individual. 0.31 Tesla MRI (Esaote, Inc., Napoli, Italy) was used in this study to evaluate water retention quantitatively in lower legs. Subjects laid on their back as shown in Figure 3.2, and a pillow was set under the upper leg and the heel to lift up the lower leg. T1 weighted imaging (T1WI) and T2 weighted imaging (T2WI) are the basic pulse sequences in MRI and the sequence weighting highlights differences in T1 and T2 relaxation time of tissue, respectively. T1WI shows fat tissue bright (high T1 signal) and water-containing tissue dark while T2WI shows tissue bright that contains water (e.g. edema, infarction). Therefore, it was chosen to be the most adequate method to evaluate water retention in this study. Furthermore, the MRI which was used in this study showed T2WI the most high-resolute imaging and capable to analysis the signal intensity. The signal intensity of T2 was analyzed at the position of the calf's maximum circumference. For the assessment of fluid retention, the T2 signal and the cross-sectional area of the lateral gastrocnemius were calculated (Figure 3.3).

Figure 3.2 Picture of the measurement during MRI.

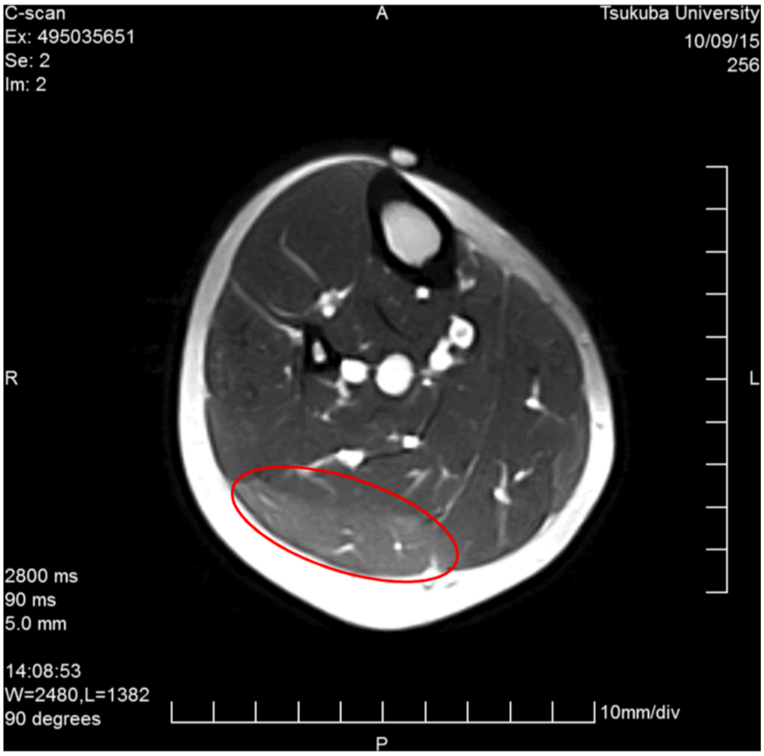


Figure 3.3 T2 signal and lateral cross-section area of the lateral gastrocnemius.

The intensity of the participants' physical activities was tracked using an activity tracker (Polar Loop, Polar Electro, Inc., Kempele, Finland) that participants wore 1 week prior to making the initial measurements through to the end of the study. Avoidance of strenuous activity was thus confirmed. The physical activity data was analyzed in 24-hour sections for every day of the 5-week study, dividing the amount of activity into 5 different degrees of exercise intensity: resting, sitting, low-, moderate-, and high-level of intensity.

Static balance ability was measured using the amplifier built-in force plate (Kistler 9286BA, Kistler Co., Ltd., Winterthur, Switzerland). Data were collected for 30 seconds and calculated the body sway from the center-of-pressure (COP). The participants had to close their eyes, cross their arms on their chest, slightly bend their hip and knee joints and keep their balance on their dominant leg for 30 seconds. The participants practiced keeping their balance for 10 seconds 3 times before every successive measurement. If the foot of the dominant leg moved laterally or if the participants opened their eyes, or their arms detached from the chest, or the nondominant leg was used to keep their balance during the measurement, they failed the test.

The static balance ability index was assigned as the "outer peripheral area" (cm2) tracing the marginal most distant balance maintaining movements, and the area of sway (rectangular) (cm2) limited by the farthest points of the individual balance maintaining body movements (Figure 3.4). Additional parameters used in the analysis were the sway area (mean circle) (cm2), the total locus length of the line tracing COP (cm), the total movement unit length, or the locus length per second (cm/sec), and the locus length per unit area (cm/cm2).

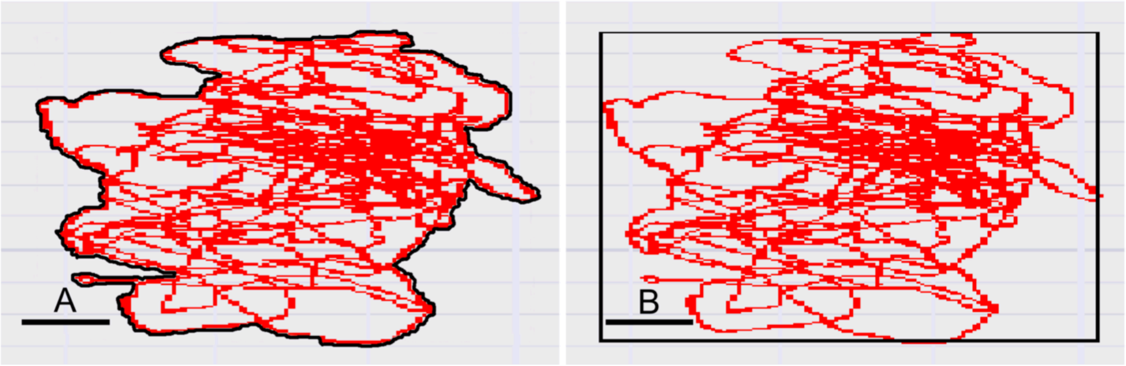


Figure 3.4 Outer peripheral area (A, cm2) and rectangular area of sway (B, cm2). Scale bar: 1 cm.

Agility was assessed using a side-step exercise, approved by the Japanese Ministry of Education Science and Technology (MEXT) as a new physical fitness test for people aged 20 to 64 (The Ministry of Education, Culture, Sports Science and Technology of Japan 1999). In this exercise, 3 tape bands are stuck on a flat floor at 1-meter intervals. From a central starting position, the participants have 20 seconds in which to step, as many times as they can, alternately on or over the outer bands, returning to the center each time. If the participant steps on or over the band, it is counted as 1. If the participant fails to reach the outer band, it is counted as 0. The test is repeated 2 times for 20 seconds with a resting time of 5 minutes. The best result of the 2 tests for each individual were recorded, according to MEXT recommendations.

Vertical jumping height was measured using a digital vertical jump assessment device (Jump-MD, T.K.K 5406, Takei Scientific Instruments Co., Ltd., Niigata, Japan). The testing belt was tightly secured around the waist and the participants were told to jump as high as possible. The principle of the vertical jumping height test is similar to that of the agility test, albeit in different planes; with agility the movement is horizontal and in jumping it is vertical. The vertical jumping test was repeated twice each time, and the best result was recorded.

Ankle flexion-extension muscle strength in the dominant leg was measured by the BIODEX System 4 (Biodex Medical Systems Inc., New York, USA). Participants were in a sitting position, with their hip and the knee joints bent 90 degrees with no flexion nor extension and feet resting on a fixation plate. The thigh and the trunk of the body were immobilized with a belt and the arms were crossed at the chest. Muscle strength was evaluated by measuring the pressure on the fixation plate, at 5 seconds for flexion and 5 seconds for extension, with a 20-second rest between them. The average value from tests 2, 3, and 4, out of 5 sets, was recorded. The ankle flexion-extension test is of a different principle, where the muscle strength is evaluated and, as in previous reports where the average values of the muscle strength in different body parts was analyzed, this common principle (Phillips et al., 1996; Sarwar et al., 1996) was also followed. The first and the last tests were excluded, as the individuals were not familiar with such tests and could avoid pressing the plate with 100% of their muscle strength in test 1 and could develop fatigue in test 5, unduly influencing the overall test results.

4-2-4. Statistical analysis

The repeated measures analysis of variance (RMANOVA) to evaluate differences between measured variables and correlation analysis to investigate the relationship between the T2 signal and athletic performance indexes in each of the 5 phases using SPSS version 22.0 (SPSS Inc, Chicago, IL, USA). The Bonferroni post hoc test was performed to assess the differences among the 5 phases in each measurement. Data presented represent means ± SE. $P<0.05$ was considered as statistically significant.

3-3. Results

3-3-1. Body composition and physical activity

Neither main effects nor significant changes were observed in body composition, amount of physical activity, steps, and energy expenditure over the 5 phases, suggesting that participants did not have any high intensity activities that could induce edema.

3-3-2. Serum hormones concentration

The highest levels of serum estradiol and progesterone were recorded in the early luteal phase. Serum estradiol levels decreased significantly in the menstrual phase and the follicular phase compared to the early luteal phase. Additionally, the menstrual phase estradiol level was significantly lower compared to the ovulatory phase, and the late luteal phase. The serum progesterone level decreased significantly in the menstrual phase and the follicular phase compared to the ovulatory phase, the early luteal phase, and the late luteal phase, and it also decreased significantly in the ovulatory phase compared to the early luteal phase. Although a main effect was observed in the serum aldosterone level and showed the highest values in the late luteal phase, there was no significant difference over the 5 phases (Figure 3.5).

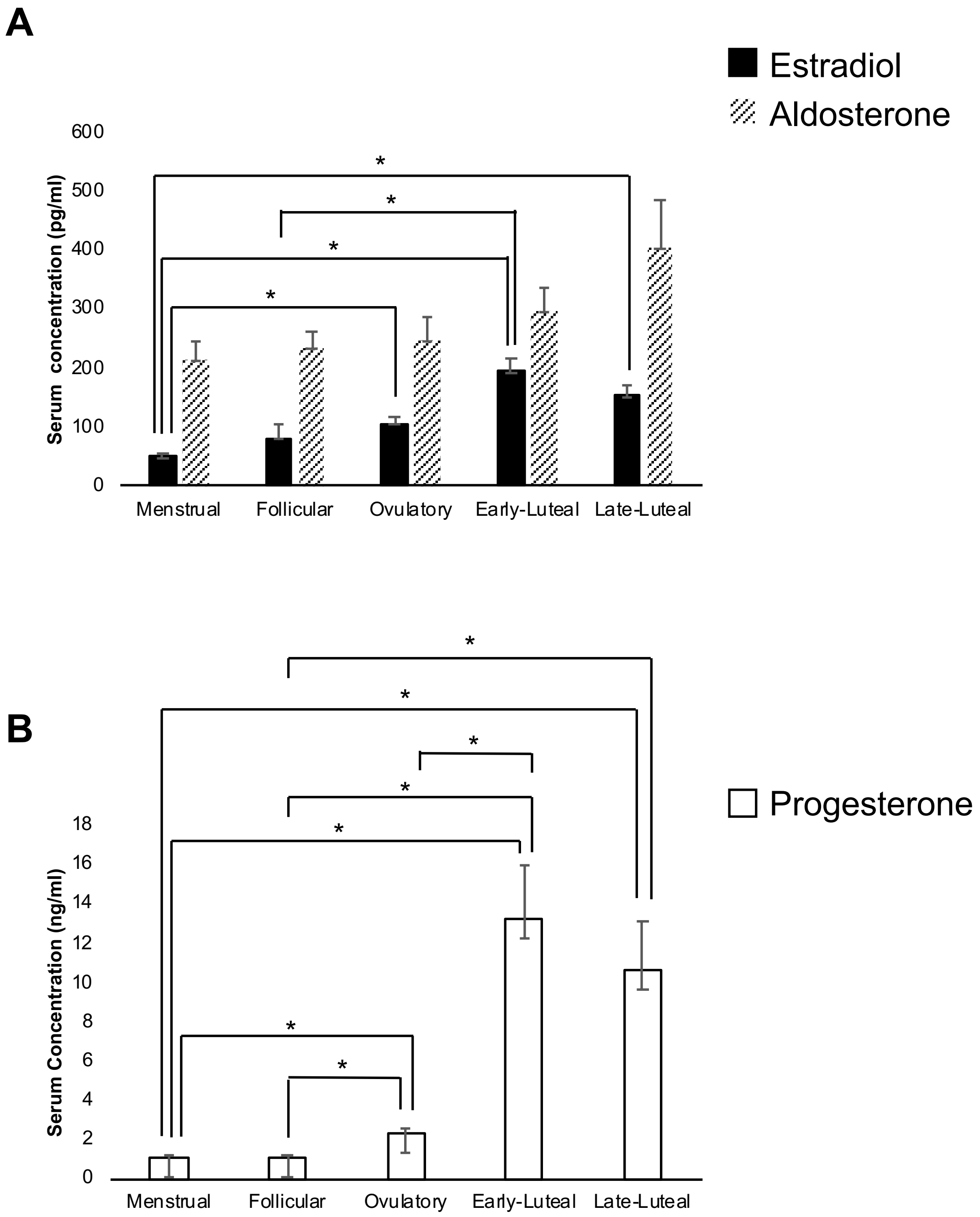


Figure 3.5 Hormone levels in blood over the 5 phases.

(A) Serum concentration of Estradiol and Aldosterone. Estradiol is represented with black bars and the Aldosterone is represented by striped bars. **(B) Serum concentration of Progesterone.**

* indicates $P<0.05$ between phases in each hormone.

3-3-3. Fluid retention in the calf

There were no significant differences in the calf circumference and the cross-sectional area of the lateral gastrocnemius in the morning (AM) and afternoon (PM), and the difference between AM and PM values over the 5 phases was also insignificant (Table 3.1). T2 signals were significantly lower in the menstrual phase in the AM compared to the ovulatory phase and were not significantly different compared to other phases. In PM, T2 signal increased significantly in the menstrual phase compared to the follicular phase, ovulatory phase, and the late luteal phase, and the difference between the AM and PM values increased significantly in the menstrual phase compared to the other 4 phases.

Table 3.1 Assessment of fluid retention in the calf.

Measurements	Menstrual cycle				
	Menstrual phase	Follicular phase	Ovulatory phase	Early luteal phase	Late luteal phase
Calf circumference (cm)					
AM	34.8 ± 0.5	34.6 ± 0.4	34.6 ± 0.5	34.6 ± 0.4	34.7 ± 0.4
PM	34.8 ± 0.5	34.6 ± 0.4	34.6 ± 0.5	34.6 ± 0.4	34.7 ± 0.4
AM-PM differ	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
T2 signal					
AM	997.7 ± 28.2^{a}	1105.8 ± 24.0	1107.3 ± 25.4^{c}	1105.1 ± 30.3	1052.3 ± 29.3
PM	1239.8 ± 35.8^{b,c,e}	1049.7 ± 38.0^{a}	1069.5 ± 32.2^{a}	1137.6 ± 27.6	1136.7 ± 34.0^{a}
AM-PM differ	242.1 ± 35.8^{b,c,d,e}	-56.2 ± 40.1^{a}	-37.8 ± 23.9^{a}	32.5 ± 35.2^{a}	84.4 ± 34.2^{a}
Cross-sectional area (mm^2)					
AM	495.4 ± 36.3	472.0 ± 33.2	466.9 ± 29.4	481.6 ± 34.5	494.8 ± 31.1
PM	466.3 ± 32.0	480.6 ± 31.4	501.3 ± 37.4	478.2 ± 29.8	503.4 ± 27.2
AM-PM differ	-29.2 ± 19.1	8.0 ± 20.7	34.4 ± 21.6	-3.4 ± 16.4	8.6 ± 14.1

The data represent means ± SEs, $P<0.05$ in a) vs. menstrual phase, b) vs. follicular phase, c) vs. ovulatory phase, d) vs. early luteal phase, e) vs. late luteal phase.

3-3-4. Athletic performance

Athletic performance data are shown in the Table 3.2. The total locus length increased significantly in the menstrual phase compared to the ovulatory phase. Other indexes of static balance ability (outer peripheral area, rectangular area, mean circle area, locus length per second, and the locus length per unit area), as well as vertical jumping ability and ankle flexion-extension muscle strength, did not change significantly over the 5 phases.

Table 3.2 Variations in the athletic performance indexes over the menstrual cycle.

Measurements	Menstrual cycle				
	Menstrual phase	**Follicular phase**	**Ovulatory phase**	**Early luteal phase**	**Late luteal phase**
Static balance ability					
Outer periphery area（cm^{2}）	14.7 ± 2.7	11.7 ± 1.2	11.0 ± 1.1	13.6 ± 1.7	13.1 ± 1.2
Rectangular（cm^{2}）	26.5 ± 5.6	20.4 ± 2.1	19.7 ± 1.9	24.0 ± 3.7	22.9 ± 2.0
Mean circle（cm^{2}）	6.2 ± 1.0	4.9 ± 0.5	4.8 ± 0.4	5.7 ± 0.7	5.3 ± 0.5
Total locus length（cm）	185.7 ± 13.3^{c}	173.6 ± 12.5	173.3 ± 14.1^{a}	180.9 ± 11.6	183.3 ± 8.8
Locus length per second（cm/sec）	6.2 ± 0.4	5.8 ± 0.4	5.8 ± 0.4	6.0 ± 0.4	6.2 ± 0.3
Locus length per unit area（cm/cm^{2}）	15.7 ± 1.5	16.4 ± 1.1	17.1 ± 1.1	15.6 ± 1.3	16.4 ± 1.2
Jump power					
Vertical jumping（cm）	38.0 ± 2.1	41.2 ± 1.7	39.8 ± 2.3	38.8 ± 1.8	38.4 ± 1.8
Agility					
Side step（point）	48.0 ± 1.4^{b,c}	51.5 ± 1.5^{a}	51.8 ± 1.5$^{a, d}$	50.1 ± 1.6^{c}	49.1 ± 1.5
Ankle flexion/extension muscle strength					
Flexion (N*M)	86.3 ± 8.5	101.1 ± 7.9	99.3 ± 7.9	100.7 ± 8.4	100.6 ± 6.1
Extension (N*M)	19.5 ± 1.4	20.2 ± 1.8	19.3 ± 2.1	18.3 ± 1.7	19.2 ± 1.3

The data represent means ± SEs, $P<0.05$ in a) vs. menstrual phase, b) vs. follicular phase, c) vs. ovulatory phase, d) vs. early luteal phase.

In the side step, the results showed a significant decrease during the menstrual phase compared to the follicular phase and the ovulatory phase. The highest score was recorded in the ovulatory phase. The values significantly decreased in the early-luteal phase compared to the ovulatory phase, though there was no significant difference between the menstrual phase and the early-luteal phase. Additionally, a negative correlation between the T2 signal changes in the AM and PM and the side step (correlation coefficient=-0.546, $P=0.025$) were found.

3-4. Discussion

3-4-1. Body composition and physical activity

Results in this study were in line with previous studies, which have reported that there was no significant difference in body composition over the menstrual cycle in participants who have normal physical activity level (Janse de Jonge 2003; Vaiksaar et al., 2011). Thus, in this study, neither physical activity nor illness affected fluid retention in the calf and any significant differences could only be attributed to menstrual cycle influence.

3-4-2. Serum hormones and fluid retention

Results in this study suggest that fluid retention and the difference between the AM and PM values are more prominent in the menstrual phase. Furthermore, the variations between the AM and PM regarding the fluid component increase gradually from the late luteal phase to the menstrual phase and then gradually decrease after the menstrual phase and continue to the follicular phase, showing less fluid variations in the ovulatory phase. MRI is considered a useful tool in detection of different pathological conditions in skeletal muscles that may cause an alteration in the signal intensity. Normal skeletal muscle MR signal intensity is usually slightly higher than the signal of water and much lower than that of fat on T1-weighted images and much lower than the signal of both fat and water on T2-weighted images. Muscle edema patterns, which almost always develop due to increased intracellular or extracellular water, are characterized by increased T2-signal intensity superimposed on normal appearance of the involved muscle or muscles (May et al., 2000).

T2WI is the one of the basic methods to show water brightness, according to the longer T2 relaxation time than other tissues due to molecular movement. Unfortunately, there was no chance to use Proton Density Weighted Imaging (PDWI) and Short-T1 Inversion Recovery (STIR) in this study using MRI. PDWI is an image produced by controlling the selection of scan parameters to minimize the effects of T1 and T2, resulting in an image dependent primarily on the density of protons in the imaging volume. STIR is typically used to null the signal from fat, and both PDWI and STIR are considered to assess the water retention more precisely. While T2WI was used to assess water retention quantitatively in this study, it is also known to visualize the fat tissue as bright which suggests a possibility of counting the T2 signal intensity not only as water but also as fat tissue in the gastrocnemius. However, the whole / lower leg body fat mass and body fat percentage did not vary significantly over the menstrual cycle in this study. Therefore, it was considered that the significant variation of the T2 signal intensity seen in menstrual phase (both in AM, PM, and between them) were due to water retention.

In previous reports, edema was assessed by measuring calf circumference or by subjective evaluation (Young et al., 1987; Sudo et al., 2010; Taylor 1979). In these reports, fluid retention, assessed using a subjective scale, peaked on the first day of menstruation (Taylor 1979; White et al., 2011), which agrees with the results in this study. Both plasma and extracellular fluid have been reported to be impacted by the menstrual cycle (Stachenfeld 2014) and can be easily expected to be affected by the ovarian hormones. Estrogen is reported to increase plasma volume by regulating the blood albumin level and leading the transition of interstitial fluid to plasma. Therefore, the extracellular fluid does not seem to increase under the independent elevation in Estrogen. Progesterone is reported to compete with aldosterone for the mineralocorticoid receptor in the distal tubule and leads renin, aldosterone, and angiotensin II to increase concomitant to the elevation in progesterone. Similarly to estrogen, independent elevation of progesterone does not play a role in water regulation. However, it has been reported that elevation of plasma volume and extracellular fluid occurs when both estrogen and progesterone level are high (Stachenfeld 2005) due to the acceleration of sodium reabsorption (Stachenfeld and Taylor, 2005). New findings in this study also support the suggestion that the edema could be the result of a delayed response to previous higher hormone levels (White et al., 2011). The reason for this still remains unclear; however, the increase in the serum hormone levels and the appearance of the edema with a 3-4 days delay is obvious. In this study, it was not possible to evaluate the intracellular and extracellular water retention by using MRI.

Although participants of the present study did not complain of any typical PMS symptoms, the results show that fluid retention increased significantly during the menstrual phase. Thus, edema occurred regardless of subjective symptoms in this study. Furthermore, T2 signal changes during the menstrual cycle showed fluid retention when the levels of estrogen, progesterone, and aldosterone were the lowest, which contradicts the main theory of premenstrual edema.

In this study, the increment of the water volume was seen in the menstrual phase, which is the phase where both ovarian hormones are at low levels. Furthermore, the water volume in the menstrual phase (AM) did not show a significant difference to the other phase's AM. A decrease in physical activity level may be one of the reasons for the occurrence of edema although, during the present study, there was no significant difference in the physical activity level over the menstrual cycle. There is a possibility that participants may unconsciously have had a decrease in physical activity levels due to menstruation. Saito et al reported that over 60% of the female participants in their study complained of lethargy during menstruation and over 40% answered that they became depressed (Saito et al., 2005). In this study, the data on specific symptoms (PMS) were collected over the menstrual cycle without assessing the mood component. Nevertheless, it is possible that related mechanisms somehow influence both mood and fluid retention during the menstrual phase.

3-4-3. Athletic performance

Hayashi et al reported that static balance ability decreased significantly in the menstrual, early-luteal, and late-luteal phases compared to the follicular phase and the locus length per second decreased significantly in the late-luteal phase (Hayashi et al., 2004), suggesting the secretion of estradiol and progesterone as a possible cause. These hormones are also reported to have a central nervous system effect, potentially affecting posture control indirectly (Posthuma et al., 1987; Lebrun 1994; Woolley 1999). In the present study, only one additional parameter in the static balance ability decreased significantly in the menstrual phase without significant changes in the late-luteal phase. It was speculated in this study that the menstrual bleeding itself might also be the reason for the impaired static balance ability. Moreover, no correlation was found between fluid retention and static balance ability.

Giacomoni et al (2000) showed that jumping power decreased significantly in the menstrual phase in athletes using the multi-drop jump and the squat jump. These tests were not carried out as they carry some risk of injury for non-athletes. However, even with a digital vertical jump assessment device it was quite difficult for participants to jump accurately and vertical jumping could also depend on arm movement much more than lower extremity mechanical power (Shetty et al., 1989), which could account for the results seen in this study.

Lebrun et al (1993) reported that the best agility performance was generally recorded in the first postmenstrual days, with the worst performance during the premenstrual interval and the first few days of the menstrual flow, which agrees with the results seen in this study. Hashimoto et al (2001) assessed agility in female handball players and reported that the agility was lowest in the menstrual phase and best in the follicular phase. However, the 25m shuttle run they used needed technical skills and no measurements were done in the ovulatory, early-luteal, and late-luteal phases. In Japan, the side step exercise is generally used in physical education classes to assess agility, was familiar to the participants of this study and they had a practice session before the measurement. Therefore, it is suggested that the results of this agility test were influenced only by the menstrual cycle itself.

Some previous reports have shown a relationship between muscle strength in the legs and the menstrual cycle in athletes, in which muscle strength increased mostly during the follicular phase (Petrofsky et al., 1976; Phillips et al., 1996; Sarwar et al., 1996; Elliott et al., 2003). The results in this study were in line with the study by Jonge et al (2001), who found no significant differences in knee flexion-extension muscle strength (isokinetic) over the menstrual cycle in women with normal physical activity levels. Thus, it is suggested that the difference in the physical activity level in participants (athletes versus non-athletes) may lead to a difference in muscle strength over the menstrual cycle.

This objective study is the first to investigate that body fluid accumulation varies over the 5 menstrual phases in spite of the lack of subjective feelings of swelling. Although there was no significant correlation between the results in the MRI T2 signal and the ovarian hormone levels, there was a significant difference over the 5 phases in diurnal body fluid variation which was evaluated by the MRI T2 signal. It iss speculated that fluid accumulation occurrences are directly or indirectly affected by the ovarian hormones and may negatively influence both athletic performance ability (especially agility which was assessed by the side-step in this study) and musculoskeletal treatment of injuries in the clinical setting. Although the study was, for practical purposes, restricted to the gastrocnemius, evaluation of hormonal effect on fluid retention within muscle tissue is highly important clinical knowledge because there could be implications in treating muscular injuries that may be overlooked by subjective examination. That is to say, even if the patient is not aware of edema, the physician can ask about menstrual status and will now have objective measuring tools to check for any edema that could affect subsequent clinical treatment options (Hess 2011).

Considering possible limitations of this study, a larger number of subjects would be beneficial for more precise evaluation of the studied parameters, though considering the novelty of the approach it have been proposed, the early stage work in this study may lead to further development of the method and an increase in the number of future studies. Longer periods of hormone level evaluation and test exercises would provide data for multiple menstrual cycles for more sustained analysis. The prevalence of menstrually related muscle edema is familiar in female athletes though the description of such edema and data on its influence on the athletic performance is lacking. In female athletes, it may be hard to discriminate whether the variation of water retention occurs due to either sex hormone fluctuations or as a response to exercises and, at this stage, healthy individuals were included in this study. Recruitment of female athletes along with women with normal physical activity levels will be favorable for this project, which at the moment is a first step in accumulating base-line data for further investigation of the phenomenon in athletes.

In summary, fluid retention in the legs (T2 signal intensity) increased significantly during the menstrual phase in the afternoon and occurred regardless of subjective symptoms. Side-step ability showed a significant decrease during the menstrual phase and had a negative correlation with fluid retention (T2 signal intensity) in the legs. These might be factors that negatively influence the physical performance of eumenorrheic women and should be considered in further sports-related studies.

4. Irregular menstrual status is related to competitive level and sports type in Japanese collegiate athletes

4-1. Introduction

Athletes rely on regular and constant physical training to build and maintain stamina and skill but the training requirements of high-intensity sports put them at a significant risk of microtrauma (Wiese-Bjornstal 2010). However, as improvement in athletic performance is highly correlated with training load (Laursen 2010; Foster et al., 1996), athletes suffering from non-mobility-threatening conditions (*e.g.*, menstruation) may feel pressured to continue their high workload, leading to a significant risk of injury in both male and female athletes. Such a connection between training intensity and variation in body condition point to an intimate link between menstrual status and injury risk and may lead to influence on their athletic performance. In the Chapter 3, mild edema in the calf muscles of female athletes due to the cyclic and rapid estradiol and progesterone fluctuations led to reductions in static balance ability and agility in eumenorrheic women. However, the influence on athletes with menstrual dysfunction have not been cleared. Previously, the connection between menstrual dysfunction and musculoskeletal injury and eating disorders in female athletes has been investigated and reported as the female athlete triad (FAT). FAT is a unique combination of eating disorders, amenorrhea and osteoporosis in female athletes and results in low energy, functional hypothalamic amenorrhea, and isolated or combined osteoporosis (Nattiv et al., 2007). However, data on collegiate female athletes is lacking, with only few reports on small groups of Japanese students or students in other countries (Komaki et al., 2001; Okano et al., 2005; Kikuchi et al., 2008). There have also been no reports linking FAT to the competitive level of Japanese athletes; higher competitive requirements at the college level (or above) can be reasonably expected to produce more intensive training requirements and exacerbate the effect of injury risk in female athletes affected by hormone fluctuations (Lebrun et al., 2002; Brunet 2005; Nichols et al., 2006). Therefore, the aim of this study was to investigate correlations between the individual risk factors for FAT and sport intensity in Japanese college athletes. Additionally, the parameters were extended to include competitive level under the hypothesis that higher competitive levels will relate to the higher menstrual irregularities, injury risk and nutrition intake.

4-2. Materials and Methods

4-2-1. Subjects and data collection

From April to May 2017 a specific questionnaire developed originally was distributed among the collegiate athletes at the University of Tsukuba and the Japan Women's College of Physical Education. All questionnaires were collected at the same time point and were completely filled in by 551 female collegiate students, including 531 individual athletes. A control group consisted of 20 students (2.3%) with no sports activity experience since elementary school. Prior to questionnaire distribution, general instructions were given to each participant. The athletes answered on their menstrual status, recent and past injury history, meal attitude, as well as other demographic information.

This study was in accordance with the latest revision of Declaration of Helsinki and was approved by the Ethics Committees of both the University of Tsukuba (approval #28-85) and the Japan Women's College of Physical Education (approval #2016-23).

4-2-2. Experimental design

4-2-2-1. Menstrual Status

Menstrual status questions included menarche age, current menstrual status (a: recently had a regular menstrual cycle, b: delayed for ~ month(s)), current menstrual cycle (a: the cycle is between 25 to 38 days, b: the cycle is less than 24 days, c: the cycle is more than 39 days, d: the cycle duration is over ±7days), past menstrual status (a: having a cyclic menstruation from menarche till now, b: experienced a delay of any kind up to 1 to 2 months ago, c: experienced a delay of any kind more than 3 months ago), experience of taking oral contraception (a: taking it now, b: have an experience in the past though not taking recently, c: never had it).

4-2-2-2. Musculoskeletal injury

Musculoskeletal injury was defined as an injury (either from direct trauma or overuse) which was the direct result of sports participation and resulted in a training stoppage of more than 3 days. The questionnaire included details of prior musculoskeletal injuries such as date of the injury occurrence, time lost from practice or competition (days), body part injured, history of stress fractures, menstrual status at the time of stress fractures (a: had menses every month, b: had menses with irregular cycle, c: menses delayed more than 3 months).

4-2-2-3. Meal attitude

Meal attitude were analyzed as frequency and nutritive choice of meals per day (a: always eat three times per day and well-balanced, b: always eat three times per day though not always well-balanced, c: usually eat three times per day though not always, d: usually eat less than two times per day), body weight reduction (a: intentional weight loss of more than 5kg, b: intentional weight loss of 1 to 4kg, c: never experienced an intentional weight reduction).

4-2-2-4. Demographic Information

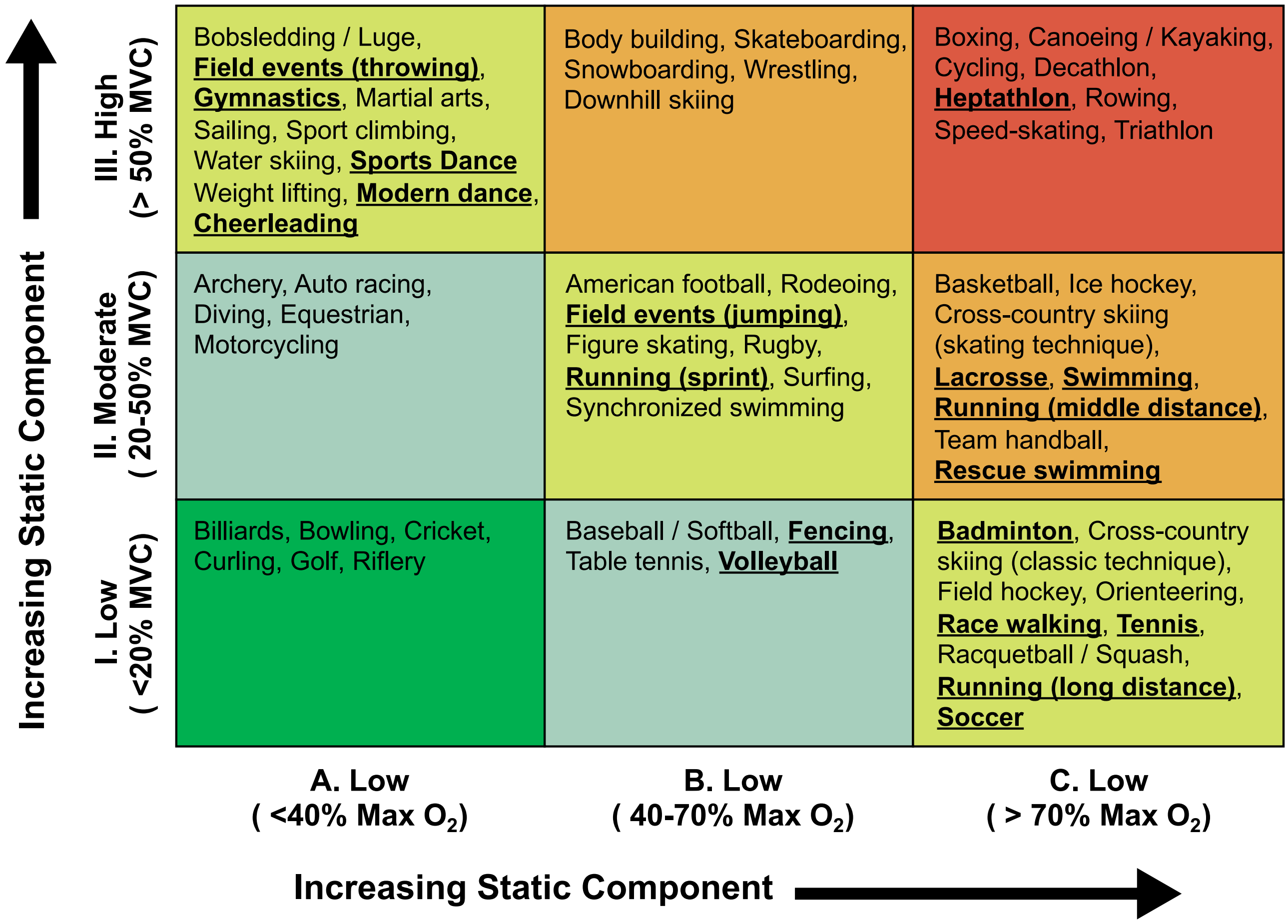
Demographic information questions addressed age, height, weight, sport type, training volume (training hours per week), the highest recent or past competitive level (a: national team level, b: national convention level, c: regional convention level, d: sub-regional convention level), years of experience and the names of any and all sports experienced in elementary school, junior high school, high school, and college. Sports that were done in gym or physical education classes were excluded as activities prescribed by the nationalized Japanese curriculum served as a common baseline for both the experimental and control groups.

4-2-3. Sports Intensity Classifications

FAT have been reported to relate to the sports type classification though the relationship with the sports intensity have not been cleared which can be reasonably expected to produce more intensive training requirements and exacerbate the effect of injury risk in female athletes affected by hormone fluctuations. Therefore, to investigate the effect of intensity level to FAT risk, the sports type classification by Jere and colleagues (Mitchell et al., 2005) were used to divide all athletes into 9 groups (Figure 4.1). Cheerleading, modern dance, and sports dance (which were not included in the Jere classification) have been reported to require higher degrees of flexibility, strength, coordination, and physical fitness levels (Padfield et al., 1993;

Thomas et al., 2004; Goodwin et al., 2004; SooHoo et al., 2005; Koutedakis et al., 2007). Additionally, rescue swimming (also not included in the Jere classification) is recognized as an official competitive sport that combines such elements as swimming and running, with previous reports showing rescue swimming velocity matching competitive swimmers in the first 50m of freestyle swimming (Alfaro et al., 2002). Therefore, cheerleading, modern dance, and sport dance were decided to classify to Group IIIA (similar to gymnastics) and rescue swimming to group IIC (similar to swimming) (Figure 4.1). Competitive levels were divided into 2 groups: “high competing level” to represent national team and national convention level, and “low competing level” which stood for the regional and sub-regional convention levels. With respect to current menstrual status and cycle regularity, these categories were divided into 2 groups, including “On Time” (recent menses) or “Delayed” (menstrual delay of 40 or more days) with the additional qualifier of “Regular “(cyclic menstruation every 25 to 38 days) and “Irregular” (irregular cycle)”. In cases of amenorrhea the previous menstrual status were subdivided into 2 groups: “Yes” (menstrual delay of greater than 3 months) and “No” (menstrual delay of less than 2 months).

Sports type which requires or prefers lean body mass where selected from the sports intensity group and compared. Race walking and Running (long distance) from Group IC, Field events (jumping) and Running (sprint) from Group IIB, Running (middle distance) from Group IIC, Gymnastics, Modern dance, Cheerleading, Sports dance from Group IIIA were selected and compared to each questions among FAT.



Increasing Static Component	A. Low (<40% Max O_2)	B. Low (40-70% Max O_2)	C. Low (> 70% Max O_2)
III. High (> 50% MVC)	Bobsledding / Luge, **Field events (throwing)**, **Gymnastics**, Martial arts, Sailing, Sport climbing, Water skiing, **Sports Dance** Weight lifting, **Modern dance**, **Cheerleading**	Body building, Skateboarding, Snowboarding, Wrestling, Downhill skiing	Boxing, Canoeing / Kayaking, Cycling, Decathlon, **Heptathlon**, Rowing, Speed-skating, Triathlon
II. Moderate (20-50% MVC)	Archery, Auto racing, Diving, Equestrian, Motorcycling	American football, Rodeoing, **Field events (jumping)**, Figure skating, Rugby, **Running (sprint)**, Surfing, Synchronized swimming	Basketball, Ice hockey, Cross-country skiing (skating technique), **Lacrosse**, **Swimming**, **Running (middle distance)**, Team handball, **Rescue swimming**
I. Low (<20% MVC)	Billiards, Bowling, Cricket, Curling, Golf, Riflery	Baseball / Softball, **Fencing**, Table tennis, **Volleyball**	**Badminton**, Cross-country skiing (classic technique), Field hockey, Orienteering, **Race walking**, **Tennis**, Racquetball / Squash, **Running (long distance)**, **Soccer**

Figure 4.1 Classification of sports based on peak static and dynamic components achieved during competition. (Mitchell et al., 2005)

4-2-4. Statistical Data Analysis

SPSS version 24.0 (SPSS Inc, Chicago, IL, USA) and the one-way ANOVA with Bonferroni post hoc test were used to evaluate differences between each of the quantitative indexes in classification groups, current menstrual cycle, mealtimes, and weight reduction. Pearson's correlation coefficient was used to investigate the relationship in the quantitative index in each classification group. Non-paired t testing was performed to evaluate differences between the quantitative index and competitive levels, current menstrual statuses, histories of amenorrhea, histories of musculoskeletal injury, and histories of bone stress fractures in each sports type. One-way ANOVA with Bonferroni post hoc test was performed to evaluate differences between the quantitative index and mealtimes and weight reduction in each sports team.

Chi-square testing was used to determine the relationship between nominal variables in all athletes, groups and each sports type. Analysis between competitive levels, current menstrual statuses, histories of amenorrhea, mealtimes, weight reduction, histories of injuries, and histories of bone stress fractures were all compared, and Fisher's exact test was also performed in a small sample size. Mean values of age, height, weight, menarche age, training hours per week, and starting age are shown ± SD and alpha values of less than 0.05 were considered significant.

Within each sport, comparisons were made between low and high competition status while additional analyses (with respect to competitive level) looked at comparisons of each sport type to its group as well as between each of the groups. For low-to-high competitive (LTH) comparisons within each sport type, no normalization was done. However, for sport-to-group (STG) comparisons, the specific sport's results (average LTH values) were normalized to the average LTH scores of that entire group. For group-to-group (GTG) comparisons, average LTH values were used. All values reported were mean ± SD and alpha values of less than 0.05 were considered significant.

4-3. Results

4-3-1. Classification results, demographics and the number of athletes meeting one or more of the FAT criteria

The results of the classification and demographics of athletes who returned the questionnaire is shown in Tables 4.1, 4.2 and 4.3. Group IIC had the highest intensity within all sports and Group IIIA had the highest training volume. As for FAT criteria, 270 (49%) athletes had menstrual dysfunction, 15 (2.7%) had low energy availability, and 108 (20%) had low bone density. These athletes therefore met only one of the three criteria. As for two of the three criteria, 58 (11%) athletes had both menstrual dysfunction and low bone density, 3 (<1%) had both menstrual dysfunction and low energy availability and 1 (<1%) had both low energy availability and low bone density. There were only 4 (<1%) athletes who met all 3 criteria.

4-3-2. High intensity sports delay menarche and contribute to menstrual irregularities in Japanese college athletes

Training volume in each group and sports are shown in Figure 4.2 and Figure 4.4. Age of menarche was significantly lower in the control group compared to Groups IB, IIB, IIC, and IIIA while Group IIIA was significantly higher than Groups IB, IC, IIB, IIC, and control group (Table 4.1 and Figure 4.3). There was a significant negative correlation between the age of menarche and the age of starting sports as well as weight among all athletes

(rs=-0.099 and -0.114, respectively) (Table 4.2), which was also seen only in Group IIIA (rs=-0.230 and -0.181, respectively) (Table 4.3).

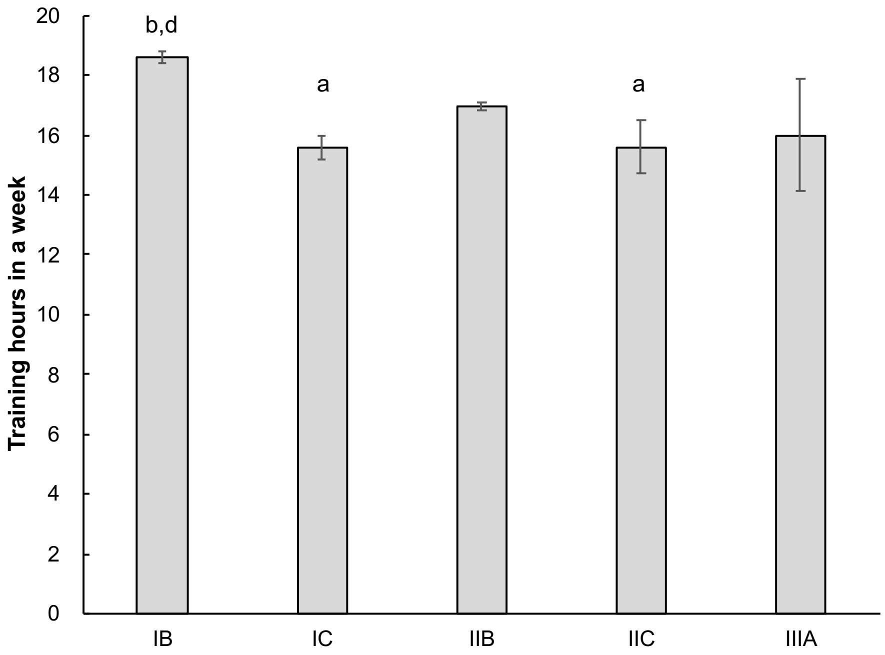


Figure 4.2 Comparison of training hours by sports type classification followed by Jere, 2005.

Training hours per week were compared between Groups IB, IC, IIB, IIC, and IIIA. a) P <0.05 in Group IB, b) P <0.05 in Group IC, and d) P <0.05 in Group IIC.

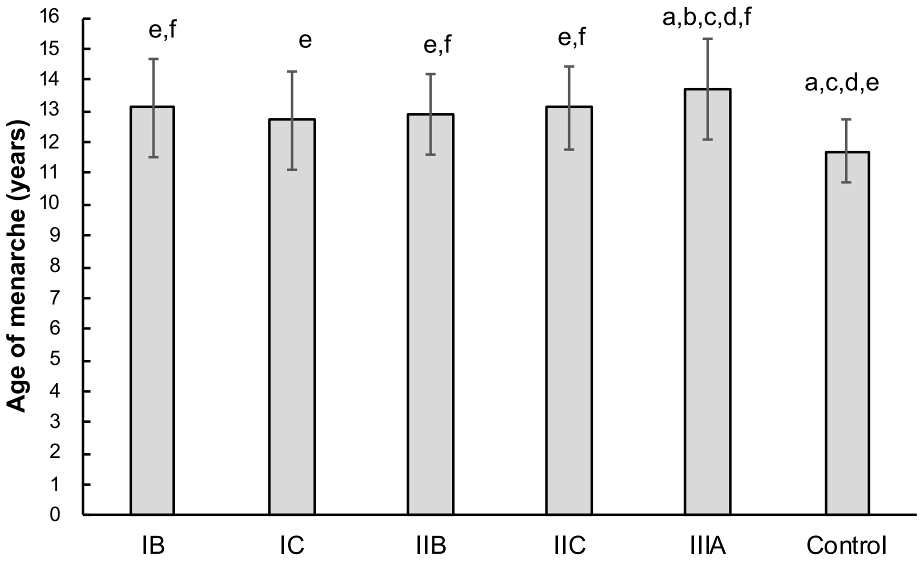


Figure 4.3 Age of menarche in each group

P<0.05 are shown as a) compared with Group IB, b) with Group IC, c) with Group IIB, d) with IIC, e) with Group IIIA, f) with Control.

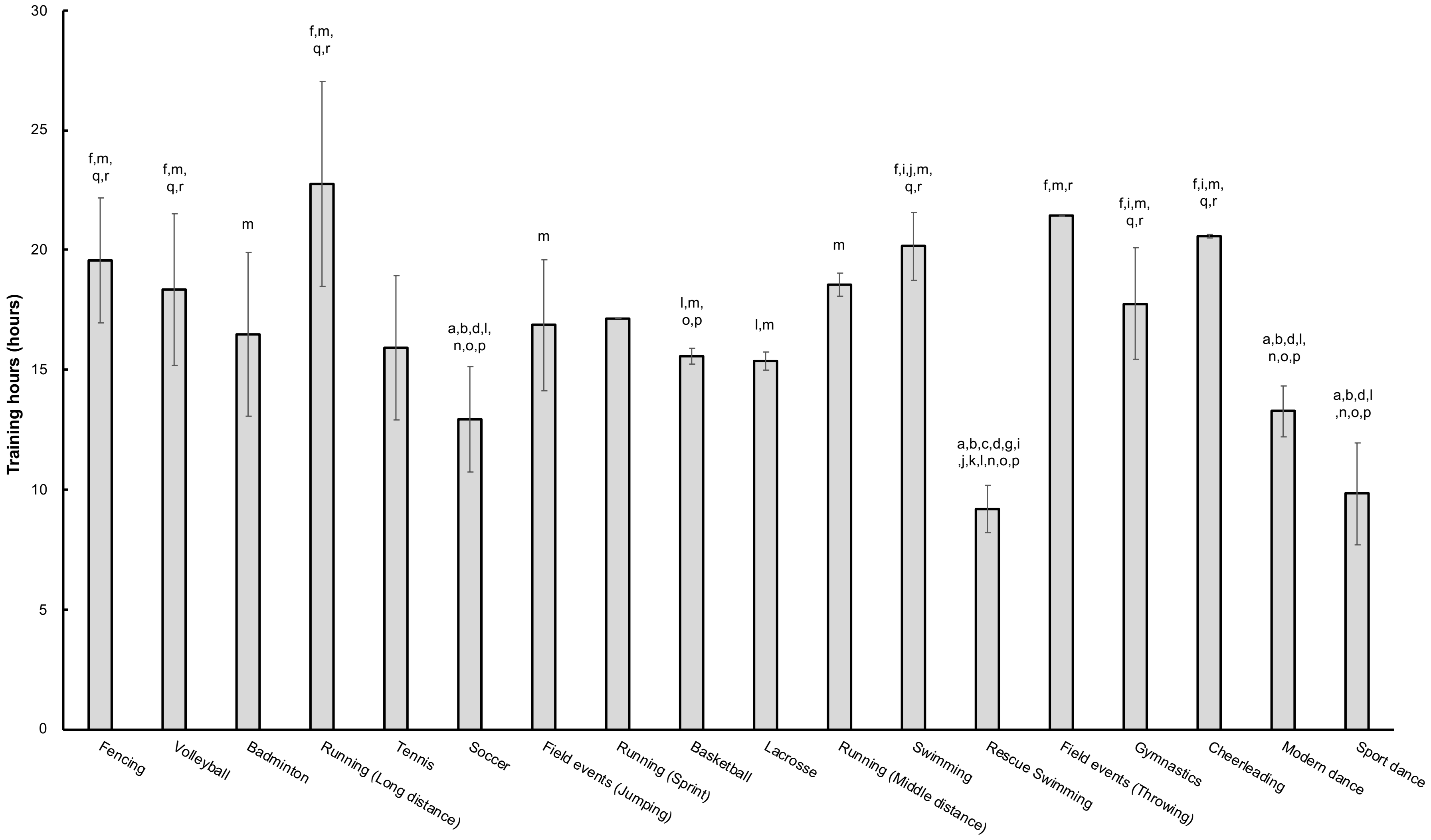


Figure 4.4 Training volume (training hours per week) in each sports team.

$P<0.05$ is found when compared with a) fencing, b) volleyball, c) badminton, d) running (long distance), f) soccer, g) field events (jumping), i) basketball, j) lacrosse, k) running (middle distance), l) swimming, m) rescue swimming, n) field events (throwing), o) gymnastics, p) cheerleading, q) modern dance, and r) dance sport.

Table 4.1 Demographic information of athletes and control

	Number of athletes			Age (years)	Height (cm)	Weight (kg)	Age of menarche (years)	Training volume (hours / week)
	All	High competing level	Low competing level					
Group IB								
Fencing	14	14	0	19.3±0.5	159.3±4.8	55.2±5.7	13.1±1.9	19.6±2.6
Volleyball	65	58	7	19.6±1.1	164.3±6.6	60.3±7.3	13.0±1.6	18.4±3.2
All	79	72	7	19.5±1.0	163.4±6.5^{b,e}	59.4±7.3^{b,c,d,e,f}	13.1±1.6^{e,f}	18.6±0.2^{b,d}
Group IC								
Badminton	17	6	11	20.1±1.0	159.3±3.9	54.4±4.4	12.5±1.3	16.5±3.4
Running (long distance)	8	1	7	19.9±0.8	157.0±2.5	47.7±5.4	13.3±1.5	22.8±4.3
Race walking	1	1	0	19.0±0	149.7±0	47.6±0	12.0±0	18.0±0
Tennis	11	0	11	19.6±1.4	160.0±4.5	55.5±5.5	12.2±1.0	15.9±0
Soccer	32	2	30	19.6±1.0	158.7±4.1	53.0±4.2	12.9±1.9	12.9±2.2
All	69	10	59	19.7±1.0	158.7±4.1^{a,c,d}	53.1±5.0^{a,d}	12.7±1.6^{e}	15.6±0.4^{a}
Group IIB								
Field events (jumping)	30	18	12	19.4±0.9	164±7.1	53.8±5.6	12.9±1.3	16.9±2.7
Running (sprint)	7	4	3	19.9±1.3	162.6±5.1	52.5±6.0	12.9±1.5	17.1±2.7
All	37	22	15	19.5±1.0	163.7±6.8^{b,e}	53.6±5.6^{a,d}	12.9±1.3^{e,f}	16.9±0.1
Group IIC								
Basketball	113	27	86	19.7±0.9	163.2±5.7	58.9±5.4	13.1±1.3	15.8±4.0
Lacrosse	29	5	24	19.9±0.9	160.3±5.0	54.3±4.9	13.3±1.3	15.4±4.3
Running (middle distance)	8	2	6	19.9±1.0	159.9±3.9	50.9±3.7	13.1±1.5	18.8±6.1
Swimming	33	26	7	19.7±0.7	160.5±5.5	54.3±5.9	13.0±1.1	20.8±8.5
Rescue Swimming	25	7	18	20±1.1	159.7±5.4	57.a±6.7	12.9±1.6	9.4±4.8
All	208	67	141	19.8±0.9	161.8±5.7^{b,e}	57.0±6.0^{a,b,c,e,f}	13.1±1.3^{e,f}	15.6±0.9^{a}
Group IIIA								
Field events (throwing)	7	3	4	19.1±0.7	162.5±3.5	66.5±7.5	12.1±1.5	21.4±2.4
Gymnastics	43	22	21	19.7±1.0	153.9±4.3	50.7±4.0	14.5±1.4	19.9±1.1
Cheerleading	24	19	5	19.5±0.8	157.7±5.2	53.9±6.8	13.5±1.6	20.6±1.6
Modern dance	40	32	8	19.4±1.1	159.0±4.9	51.1±5.5	13.4±1.6	13.4±6.0
Dance sport	20	9	11	19.8±0.6	159.2±4.2	52.0±4.8	13.4±1.1	11.4±9.9
All	134	85	49	19.6±0.9	157.4±5.2^{a,c,d}	52.4±6.3^{a,d}	13.7±1.6^{a,b,c,d,f}	16.0±1.9
Group IIIC								
Hepathlon	4	4	0	19.8±0.5	166.2±6.3	59.3±4.2	12.3±1.7	18.8±0.5
All athletes	531	258	273	19.7±1.0	160.7±6.1	56.5±3.9	13.1±1.5	16.8±1.6
Control	20	-	-	20.9±1.7^{a,b,c,d,e}	159.7±4.5	51.9±5.1^{a,d}	11.7±1.0^{a,c,d,e}	-

$p<0.05$ with a) Group IB, b) Group IC, c) Group IIB, d) Group IIC, e) Group IIIA, f) Control group

Table 4.2 Correlation of variable indexes in all athletes

	Age (years)	Height (cm)	Weight (kg)	Age of menarche (years)	Training hours (hours)	Training frequency (days / week)	Training hours in 1 week (hours)	Age starting sports (years)
Age	1							
Height	0.026	1						
Weight	-0.032	0.674**	1					
Age of menarche	0.016	-0.054	-0.114	1				
Training hours	-0.037	-0.071	-0.029	0.082	1			
Training frequency	-0.035	0.149**	0.101	0.488	0.255**	1		
Training hours in 1 week	-0.046	0.008	0.016	0.096*	0.861**	0.663**	1	
Age starting sports	0.021	0.06	0.079	-0.099*	-0.088*	-0.054	0.106*	1

*p<0.05, **p<0.01

Table 4.3 Correlation of variable indexes in Group IIIA

	Age (years)	Height (cm)	Weight (kg)	Age of menarche (years)	Training hours (hours)	Training frequency (days / week)	Training hours in 1 week (hours)	Age starting sports (years)
Age	1							
Height	0.166	1						
Weight	-0.031	0.551**	1					
Age of menarche	0.125	-0.144	-0.181*	1				
Training hours	-0.093	-0.174	0.147	0.073	1			
Training frequency	0.083	0.019	-0.02	0.051	0.482**	1		
Training hours in 1 week	-0.025	-0.128	0.071	0.083	0.912**	0.778**	1	
Age starting sports	-0.004	0.034	0.066	-0.230**	-0.149	-0.223**	0.205*	1

*p<0.05, **p<0.01

4-3-3. Highly repetitive, high training volume sports increase risk of musculoskeletal injury and high intensity sports negatively impact nutrition choices

Intensity number did not affect the number of athletes who experienced musculoskeletal injury severe enough to require rest for more than 3 days. Group IIB showed significantly higher numbers of athletes who had experienced stress fractures while Group IIC showed significantly lower athlete numbers with regard to stress fractures (p=0.038) (Table 4.4). Furthermore, group comparison which requires meal and body restriction showed that number of athletes who experienced stress fracture were significantly higher in Group IIB and IIC (Adjusted Residual=2.5 and 3.2, respectively).

Group IIIA reported a significantly lower number of athletes who usually have well balanced meals 3 times per day while Group IIB and IIC reported significantly higher numbers. Furthermore, in weight reduction, Group IIIA reported a significantly higher number of athletes who frequently reduce their weight over 5kg or around 1-2kg for improvement of sports performance (Table 4.4). Furthermore, group comparisons which require meal and body restriction showed that the number of athletes in Group IIIA was significantly worse in those who usually have well balanced meals 3 times per day (Adjusted Residual=-3.0), and was significantly higher in athletes who experienced amenorrhea (Adjusted Residual=5.1).

Table 4.4 Relationships by sports classifications

			Group					
			IB (n=79)	IC (n=69)	IIB (n=37)	IIC (n=208)	IIIA (n=134)	Control (n=20)
Experience of Amenorrhea	**Yes**	**Count**	11	15	19	33	26	4
		% within Total	2.00%	2.70%	3.50%	6.00%	4.80%	0.70%
		Adjusted Residual	-1.4	0.4	5.0*	-1.8	-0.1	0
	No	**Count**	68	54	18	175	108	16
		% within Total	12.40%	9.90%	3.30%	32.00%	19.70%	2.90%
		Adjusted Residual	1.4	-0.4	-5.0*	1.8	0.1	0
Meal attitude	**3 meals/day, well balanced**	**Count**	21	25	18	81	33	4
		% within Total	3.98%	4.74%	3.42%	15.37%	6.26%	0.70%
		Adjusted Residual	-1.5	0.5	2.1*	2.2*	-2.4*	-1.3
	Just 3 meals/day	**Count**	29	26	8	48	41	4
		% within Total	5.50%	4.93%	1.52%	9.11%	7.78%	0.70%
		Adjusted Residual	1.7	1.7	-1.0	-2.2*	0.5	-0.9
	Mainly 3 meals/day, sometimes 2meals	**Count**	24	17	11	66	52	6
		% within Total	4.55%	3.23%	2.09%	12.52%	9.87%	1.10%
		Adjusted Residual	-0.4	-1.5	-0.3	-0.2	1.9	-0.2
	Mainly less than 2meals/day	**Count**	5	1	0	13	8	6
		% within Total	0.95%	0.19%	0.00%	2.47%	1.52%	1.10%
		Adjusted Residual	0.5	-1.5	-1.5	0.9	0.5	4.6*
Weight reduction	**Frequently over 5kg**	**Count**	0	1	1	2	6	0
		% within Total	0.00%	0.19%	0.19%	0.38%	1.14%	0.00%
		Adjusted Residual	-1.3	-0.3	0.4	-1.3	2.5*	-0.6
	Frequently around 1-2kg	**Count**	33	28	20	76	83	5
		% within Total	6.26%	5.31%	3.80%	14.42%	15.75%	0.90%%
		Adjusted Residual	-0.7	-0.9	1.1	-3.4*	4.4*	-1.8
	Never tried	**Count**	46	40	16	130	45	15
		% within Total	8.73%	7.59%	3.04%	24.67%	8.54%	2.70%
		Adjusted Residual	1.1	1.0	-1.2	3.7*	-5.7*	2.0*
History of stress fracture	**Yes**	**Count**	18	16	15	32	24	0
		% within Total	3.30%	2.90%	2.70%	5.90%	4.40%	0.00%
		Adjusted Residual	0.7	0.7	3.3*	-2.1*	-0.7	-2.2*
	No	**Count**	61	53	22	176	110	20
		% within Total	11.20%	9.70%	4.00%	32.20%%	20.10%%	4.50%
		Adjusted Residual	-0.7	-0.7	-3.3*	2.1*	0.7	2.2*

* $p<0.05$

4-3-4. Classification of competitive levels and numbers of athletes in each by intergroup and group-to-group comparisons

The results of LTH (Table 4.5), STG (Table 4.6) and GTG (Table 4.7-4.10) comparisons contained significant differences. In LTH (low competitive level versus high competitive level) classifications within each sport, basketball and modern dance showed significantly higher training volumes in high competitive level than in low, a trend reflected in all athletes. According to the classification of intensity level/training volumes in sport-to-group (STG) comparisons (comparisons of individual sports to the others within their respective groups), there was no significant difference in competing level and sports type in Groups IB and IIB (Table 4.6). However, within the groups, there were differences in athlete numbers with respect to competitive levels. In Group IC, badminton had a significantly higher number of athletes at the high competing levels. In Group IIC, swimming had a significantly higher number of athletes in a high competing level while basketball had a significantly higher number of athletes at a low competing level. In Group IIIA, modern dance had a significantly higher number of athletes in a high competing level while gymnastics had a significantly higher number of athletes in low competing levels. Through variable indexes, there was no significant difference seen within Group IB (Table 4.6). Group IC, IIC, and IIIA showed significant higher training volumes at the high competing level than in low. With regard to nominal indexes, no significant relationship between competitive level and other nominal indexes were seen in Groups IB, IIC, and IIIA (Table 4.7, Table 4.8). Within group-to-group comparisons (comparisons between each of the groups), a significantly higher number of athletes were in the high competitive level in Groups IB and IIIA while Groups IC and IIC were significantly lower (Table 4.9).

Table 4.5 LTH Comparison

	Competing level	Number of athletes	Age (years)	Height (cm)	Weight (kg)	Age of menarche (years)	Training hours in 1 week (hours)	Age of strating sports (years)
All	**High**	258	19.7 ± 0.9	161.1 ± 6.3	55.8 ± 7.1	13.3 ± 1.6*	18.1 ± 6.1*	7.4 ± 1.9*
	Low	273	19.7 ± 1.0	160.3 ± 5.8	55.2 ± 6.2	13.0 ± 1.4*	15.1 ± 6.1*	8.0 ± 2.1*
Volleyball	**High**	58	19.7 ± 1.1	164.0 ± 6.5	60.0 ± 7.1	13.1 ± 1.6	18.4 ± 3.2	8.1 ± 1.6
	Low	7	18.7 ± 0.8	167.0 ± 6.6	62.8 ± 8.6	12.3 ± 1.4	18.0 ± 3.5	7.7 ± 1.5
Badminton	**High**	6	19.5 ± 0.8*	159.0 ± 2.5	58.1 ± 5.4*	12.2 ± 1.7	18.0 ± 3.8	8.7 ± 0.5
	Low	11	20.4 ± 1.0	159.5 ± 4.6	52.4 ± 2.1*	12.7 ± 1.0	15.6 ± 3.1	7.5 ± 2.9
Soccer	**High**	2	19.5 ± 0.7	157.1 ± 2.7	49.7 ± 5.2	12.5 ± 0.7	12.0 ± 0	6.0 ± 0
	Low	30	19.6 ± 1.0	158.8 ± 4.2	53.2 ± 4.1	12.9 ± 1.9	13.0 ± 2.3	7.4 ± 2.0
Field events (Jumping)	**High**	18	19.3 ± 0.6	166.3 ± 7.1*	54.2 ± 6.4	13.1 ± 1.3	17.1 ± 2.5	8.0 ± 2.4
	Low	12	19.6 ± 1.2	160.6 ± 5.9*	53.2 ± 4.4	12.8 ± 1.4	16.5 ± 3.1	9.2 ± 3.6
Running (sprint)	**High**	4	19.3 ± 1.5	163.8 ± 6.1	53.6 ± 6.4	13.3 ± 1.5	17.5 ± 2.9	6.8 ± 3.6
	Low	3	20.7 ± 0.6	160.9 ± 4.1	51.1 ± 6.4	12.3 ± 1.5	16.7 ± 2.9	9.0 ± 1.0
Basketball	**High**	27	20.2 ± 1.0	164.1 ± 7.0	60.5 ± 5.5	13.3 ± 1.7	17.2 ± 3.4*	8.1 ± 1.2
	Low	86	19.6 ± 0.9	162.9 ± 5.3	58.3 ± 5.4	13.0 ± 1.1	15.3 ± 4.0*	8.1 ± 1.5
Lacrosse	**High**	5	19.8 ± 1.1	158.4 ± 3.7	54.4 ± 3.5	13.4 ± 1.5	18.0 ± 4.5	8.6 ± 2.8
	Low	24	20.0 ± 0.9	160.7 ± 5.3	54.5 ± 5.3	13.4 ± 1.3	15.1 ± 4.3	9.5 ± 2.1
Running (middle distance)	**High**	2	20.5 ± 0.7	159.0 ± 4.2	49.7 ± 2.3	13.5 ± 2.1	12.5 ± 3.5	8.5 ± 0.7
	Low	6	19.7 ± 1.0	160.2 ± 4.2	51.3 ± 4.1	13.0 ± 1.4	20.8 ± 5.4	8.5 ± 2.4
Swimming	**High**	26	19.8 ± 0.7	160.4 ± 5.5	54.3 ± 6.0	13.1 ± 1.1	22.1 ± 8.2	6.2 ± 0.8
	Low	7	19.3 ± 0.8	161.1 ± 5.7	54.1 ± 6.1	12.6 ± 1.4	15.7 ± 8.0	6.7 ± 1.5
Rescue Swimming	**High**	7	19.7 ± 1.0	160.7 ± 4.5	57.1 ± 9.3	13.1 ± 1.1	7.6 ± 4.2	7.6 ± 2.1
	Low	18	20.1 ± 1.1	159.3 ± 5.7	57.1 ± 5.8	12.8 ± 1.8	10.1 ± 5.0	8.5 ± 2.1
Field events (throwing)	**High**	3	19.0 ± 1.0	162.7 ± 3.2	71.3 ± 7.8	12.0 ± 1.0	21.7 ± 2.9	7.7 ± 2.1
	Low	4	19.3 ± 0.5	162.3 ± 4.2	62.8 ± 5.6	12.3 ± 1.9	21.3 ± 2.5	6.8 ± 1.0
Gymnastics	**High**	22	19.8 ± 1.0	155.0 ± 4.4	51.2 ± 4.3	14.6 ± 1.5	22.0 ± 9.4	5.4 ± 1.8*
	Low	21	19.6 ± 1.0	152.7 ± 3.8	50.2 ± 3.6	14.3 ± 1.3	17.7 ± 12.4	7.6 ± 2.8*
Cheearleading	**High**	19	19.6 ± 0.8	158.4 ± 4.3	53.1 ± 6.3	13.7 ± 1.6	20.5 ± 1.4	7.3 ± 2.1
	Low	5	19.0 ± 0.0	154.9 ± 7.7	57.0 ± 8.7	12.4 ± 1.1	21.0 ± 2.2	7.8 ± 2.8
Modern dance	**High**	32	19.6 ± 1.1*	159.0 ± 4.6	50.9 ± 5.2	13.3 ± 1.7	14.6 ± 5.4*	6.7 ± 1.5
	Low	8	18.6 ± 0.5*	159.3 ± 6.4	52.0 ± 6.8	13.8 ± 0.7	8.6 ± 6.2*	6.8 ± 1.4
Sport dance	**High**	9	19.0 ± 0.5	161.1 ± 4.1*	54.8 ± 4.3*	13.4 ± 1.4	14.1 ± 11.8	7.7 ± 2.2
	Low	11	19.9 ± 0.7	157.6 ± 3.6*	49.7 ± 3.9*	13.3 ± 0.9	9.1 ± 7.8	7.0 ± 1.9

* shows $p<0.05$ between High and Low in each sports type

Table 4.6 STG comparison

			Competing level		p value
			High	Low	
GroupIB	**Fencing**	**Count**	14	0	0.24
		% Within GroupIB	17.70%	0.00%	
		Adjusted Residual	1.3	-1.3	
	Volleyball	**Count**	58	7	
		% Within GroupIB	73.40%	8.90%	
		Adjusted Residual	-1.3	1.3	
Group IC	**Baminton**	**Count**	6	11	0.017
		% Within GroupIC	8.80%	16.20%	
		Adjusted Residual	3.1*	-3.1*	
	Running (long distance)	**Count**	1	7	
		% Within GroupIC	1.50%	10.30%	
		Adjusted Residual	-0.1	0.1	
	Tennis	**Count**	0	11	
		% Within GroupIC	0%	16.20%	
		Adjusted Residual	-1.4	1.4	
	Soccer	**Count**	2	30	
		% Within GroupIC	2.90%	44.10%	
		Adjusted Residual	-1.6	1.6	
Group IIB	**Field events (jumping)**	**Count**	18	12	0.606
		% Within GroupIIB	48.60%	32.40%	
		Adjusted Residual	0.1	-0.1	
	Running (sprint)	**Count**	4	3	
		% Within GroupIIB	10.80%	8.10%	
		Adjusted Residual	-0.1	0.1	
Group IIC	**Basketball**	**Count**	27	86	＜0.001
		% Within GroupIIC	13.00%	41.30%	
		Adjusted Residual	-2.8*	2.8*	
	Lacrose	**Count**	5	24	
		% Within GroupIIC	2.40%	11.50%	
		Adjusted Residual	-1.9	1.9	
	Running (middle distance)	**Count**	2	6	
		% Within GroupIIC	1%	2.90%	
		Adjusted Residual	-0.4	0.4	
	Swimming	**Count**	26	7	
		% Within GroupIIC	12.50%	3.40%	
		Adjusted Residual	6.2*	-6.2*	
	Lifesaving	**Count**	7	18	
		% Within GroupIIC	3.40%	8.70%	
		Adjusted Residual	-0.5	0.5	
Group IIIA	**Field events (throwing)**	**Count**	3	4	0.006
		% Within GroupIIIA	2.20%	3.00%	
		Adjusted Residual	-1.2	1.2	
	Gymnastics	**Count**	22	21	
		% Within GroupIIIA	16.40%	15.70%	
		Adjusted Residual	-2.0*	2.0*	
	Cheerleading	**Count**	19	5	
		% Within GroupIIIA	14%	3.70%	
		Adjusted Residual	1.8	-1.8	
	Modern dance	**Count**	32	8	
		% Within GroupIIIA	23.90%	6.00%	
		Adjusted Residual	2.6*	-2.6*	
	Dance sports	**Count**	9	11	
		% Within GroupIIIA	6.70%	8.20%	
		Adjusted Residual	-1.9	1.9	

* $p<0.05$

Table 4.7 GTG comparison in variable indexes

	Group									
	IB (n=79)		IC (n=69)		IIB (n=37)		IIC (n=208)		IIIA (n=134)	
	High	Low	High	Low	High	Low	High	Low	High	Low
Age (years)	19.6±1.0	18.7±0.8	19.5±0.7	19.8±1.1	19.3±0.8	19.8±1.2	20.0±0.1	19.7±0.1	19.6±1.0	19.4±0.9
Height (cm)	163.1±6.5	167.0±6.6	157.6±3.5	158.9±4.2	165.8±6.8*	160.6±5.5	161.7±0.8	161.9±0.5	158.2±4.8*	155.9±5.6
Weight (cm)	59.1±7.1	62.8±8.6	54.9±6.1	52.8±4.8	54.1±6.3	52.8±4.7	57.0±0.8	57.0±0.5	52.6±6.4	52.1±6.2
Trainng volume (hours/week)	18.6±3.1	18.0±3.5	16.8±3.8*	15.3±4.3	17.2±2.5	16.5±3.0	18.0±0.9*	14.9±0.4	18.0±7.7*	14.9±10.4
Age of menarche (years)	13.1±1.6	12.3±1.4	12.3±1.3	12.8±1.6	13.1±1.3	12.7±1.3	13.2±0.2	13.0±0.1	13.7±1.7	13.6±1.4
Age starting sports	8.2±1.6	7.7±1.5	8.4±1.7	7.6±2.2	7.8±2.6	9.1±3.2	7.4±0.2*	8.4±0.2	6.6±2.0	7.3±2.3

*p<0.05 between High and Low competing level in each group

Table 4.8 Competing level and the History of amenorrhea in Group IC

			Competing level	
			High	Low
History of amenorrhea	**Yes**	**Count**	9	31
		% within Group IC	13.00%	44.90%
		Adjusted Residual	2.2*	-2.2*
	No	**Count**	1	28
		% within Group IC	1.40%	40.60%
		Adjusted Residual	-2.2*	2.2*

* p<0.05

Table 4.9 Significant relationship with competing level in Group IIC

			Competing level	
			High	Low
Current menstrual cycle	**Regular**	**Count**	58	134
		% within Group IIC	27.90%	64.40%
		Adjusted Residual	-2.1*	2.1*
	Delayed	**Count**	9	7
		% within Group IIC	4.30%	3.40%
		Adjusted Residual	2.1*	-2.1*
Meal attitude	**3 meals/day, well balanced**	**Count**	36	45
		% within Group IIC	17.30%	21.60%
		Adjusted Residual	3.0*	-3.0*
	Just 3 meals/day	**Count**	14	34
		% within Group IIC	6.70%	16.30%
		Adjusted Residual	-0.5	0.5
	Mainly 3 meals/day, sometimes 2meals	**Count**	15	51
		% within Group IIC	7.20%	24.50%
		Adjusted Residual	-2.0*	2.0*
	Mainly less than 2meals/day	**Count**	2	11
		% within Group IIC	1.00%	5.30%
		Adjusted Residual	-1.3	1.3
History of injuries	**Yes**	**Count**	46	114
		% within Group IIC	22.10%	54.80%
		Adjusted Residual	-2.0*	2.0*
	No	**Count**	21	27
		% within Group IIC	10.10%	13.00%
		Adjusted Residual	2.0*	-2.0*

* p<0.05

Table 4.10 Competing level in GTG

			Groups				
			IB (n=79)	IC (n=69)	IIB (n=37)	IIC (n=208)	IIIA (n=134)
Competing level	**High**	**Count**	72	10	22	67	85
		% within Group	13.60%	1.90%	4.10%	12.60%	16.00%
		Adjusted Residual	8.2*	-6.1*	1.4	-6.1*	4.0*
	Low	**Count**	7	59	15	141	49
		% within Group	1.30%	11.10%	2.80%	26.60%	9.20%
		Adjusted Residual	-8.2*	6.1*	-1.4	6.1*	-4.0*

* P<0.05

4-3-5. Competitive level does not specifically increase menarche age at higher levels in groups, but lower competitive levels experience more amenorrhea

With respect to age of menarche, there was a general increase as competitive level increased which was seen only in overall comparisons but not in the intensity-classified groups.

Within each sport, there was no significant relationship seen between current menstrual status and competitive level. However, in comparisons through classification of intensity level groups, Groups IC and IIC showed a significant relationship between menstrual status and the competitive level (Table 4.10). The number of athletes in all groups but IIC who currently had a regular menstrual cycle were significantly higher at high competing levels while low competing level athletes were significantly higher in menstrual irregularities (including oligomenorrhea, amenorrhea, and polymenorrhea). Additionally, the number of athletes with a past experience of amenorrhea were significantly higher in lower competing levels while athletes in high competing levels were significantly lower in all athletes (Fisher’s exact test, $p<0.001$) (Table 4.11). A notable exception to this finding exists: athletes in the high competing level of Group IC had significantly higher numbers of athletes who experience amenorrhea (Fisher’s exact test, $p=0.026$) which was completely opposite to the result seen in all athletes

Table 4.11 Relationship in menstrual status and competing level

			Competing level	
			High	Low
Current menstrual cycle	Regular	Count	206	196
		% within Competing level	51.20%	48.80%
		Adjusted Residual	2.2*	-2.2*
	Irregular	Count	52	77
		% within Competing level	40.30%	59.70%
		Adjusted Residual	-2.2*	2.2*
Experience of Amenorrhea	Yes	Count	89	122
		% within Competing level	16.80%	23.00%
		Adjusted Residual	-2.4*	2.4*
	No	Count	169	151
		% within Competing level	31.80%	28.40%
		Adjusted Residual	2.4*	-2.4*

* $p<0.05$

4-3-6. Higher competitive levels do not generally correlate to musculoskeletal injuries or stress fractures and do not impact nutrition choices.

Generally, the effect of competitive level on musculoskeletal injuries, especially stress fractures, was not significant in all athletes. However, in Group IIC (Table 4.8), lower competitive levels had higher numbers of athletes with injuries, but not stress fractures, and in Group IIB (Table 4.7), athletes who did have injuries were more likely to have stress fractures. This counterintuitive relationship between low and high competitive level was only seen in Group IIC sports.

From the intensity-based classifications, Group IIC was the only group to show a significant relationship between competing level and meal attitude (Table 4.8). Athletes in the high competing level in this group had a significantly higher number of athletes who usually have well-balanced meals, while the number of athletes in low level competition significantly chose less well-balanced meals (p=0.017).

4-3-7. Competitive levels within each sport significantly affect other variables such as starting age and height

In high competitive level Group IB and IIIA sports, Group IIIA was significantly lower in starting age compared to other groups including the control group. Within Group IIIA, the starting age in artistic gymnastics was significantly lower than volleyball (IB), badminton, tennis and soccer (IC), jumping (IIB), basketball (IIC), swimming and rescue swimming (IIC), throwing (IIIA), and controls. Additionally, Groups IIB and IIIA showed a significantly higher height at the higher competing level than at the lower level.

4-4. Discussion

In this study, 531 Japanese collegiate athletes against 20 non-active control students were surveyed to investigate the risk of female athlete triad with regard to sports intensity level, sports type, and competing level. The aim was to identify which of the accepted FAT risk factors were correlated to the intensity and training volume of various sports in a Japanese collegiate female population. Additionally, as there are no current reports of the effect of competitive level on FAT risk at the college level in Japan, the second aim was to see how this variable impacted FAT risk factors in the respondents.

Comparisons across different sports can be treacherous; however, similarities in movement, objective and kinesthetic demand can be exploited to create groups of roughly similar sports. Advantage of this were taken by grouping the sports according to the Jere classification method (Mitchell et al., 2005). The effect analyses with regard to intensity and training volume were then able to see if correlations with FAT risk factors existed. However, to avoid bias from the limitations of group-to-group comparisons, Comparison at each sport to the others within its group were looked at and added a comparative category in which competitive levels (low or high) in each sport could be analyzed for links to FAT risk factors. The results in sports intensity and training volume are in line with other reports. One reported that the training volume (hours per week) was highest in athletes who are competing in aesthetic sports (which includes Group IIIA in this study) compared to power, technical, anti-gravitation, and ball game sports (Torstveit et al., 2005). However, Group IIB sports types (which showed the highest training volume in the study) were classified into different groups in this previous report, showing swimming and lifesaving as anti-gravitation sports, basketball as ball game sports (Torstveit et al., 2005). Group IIIA showed a significantly higher age of menarche compared to other groups, including controls, and the control group was significantly lower than Groups IB, IIB, IIC, and IIIA. These results are in line with previous reports that the average menarche age in athletes is higher than non-athletes (Malina et al., 1978). With regard to menarche age by sport types, the earliest was tennis (12.2±1.0 years old) while the latest was artistic gymnastics (14.5±1.4 years old). These aesthetic sports are high intensity sports which place excessive demands on athletes to maintain low body fat, weight restriction, and heavy intensity training from a very young age (Baxter-Jones et al., 1994; Dušek 2001 and 2004; Redman et al., 2005). Similarly, a study in female top competing level athletes reported that the menarche age of athletes in aesthetic sports (14.5±2.0 years old) was higher than other sports type such as technical skill/ endurance sports, ball sports, power sports, and weightlifting sports (Nose et al., 2014).

In this study, the starting age in artistic gymnastics (Group IIIA) was significantly lower than volleyball, field events (jumping), basketball, lacrosse, and lifesaving, while cheerleading and sports dance were significantly higher than lacrosse, and modern dance was significantly lower than field events (throwing), basketball, lacrosse, which strongly supports information from previous reports (Malina 1983 and 2010). Aesthetic and near-aesthetic sports such as artistic gymnastics, rhythmic gymnastics, dance, figure skating, modern dance, as well as swimming are generally known as “early-entry sports” (Malina 1983 and 2010) which typically require early specialization and high intensity during prepubescent stages (Myer et al., 2015). Group IIB (which includes jumping and sprint) showed a higher level of amenorrhea compared to other groups. There are some reports on sports that require horizontal (*e.g.,,* running and long jump) or vertical (*e.g.,,* high jump, gymnastics) movements of the body where excessive fat mass is considered a disadvantage (Olds et al., 1993; Sudgot-Borgen et al., 2010) which may lead to low body fat, weight and lead to menstrual irregularities (Torstveit et al., 2005). As a highly dynamic sport, it could negatively affect regular menstrual periods, especially in young Japanese women (Stefani et al., 2016).

Taken together, these results indicate a significant relationship between intensity and delay of menarche; training volume is less a factor than the actual intensity of the sport type itself. Interestingly, starting age is negatively correlated to menstrual irregularities, meaning that intensity of the sport type is more important.

With regards to injuries, both the intensity of training and the amount of time training may affect susceptibility, even without menstrual irregularities. Musculoskeletal injuries may be either traumatic (broken bones, torn muscles) or chronic injuries brought about by repetitive motion and insufficient recovery time. Sports which are relatively restricted in dynamic movement (running, jumping, etc) were found to be significantly higher reporting of severe musculoskeletal injuries. Additionally, the overwhelming type of injury for athletes who have experienced severe musculoskeletal injury requiring more than 3 days of rest was found to be the stress fracture (Fisher's exact test, $p<0.01$), which is highly indicative of repetitive motion injury. In almost all sports, with the exception of the heptathlon, the range of motion is restricted by the demand of the sport itself and it is possible that the cross-training schemes of heptathletes is somehow protective against stress fractures. This data indicates that, in sports with lower dynamic movement demands, cross-training methods (*e.g.*, plyometrics) involving complimentary musculoskeletal groups may serve a protective role against repetitive motion injuries.

Nutrition is the cornerstone of sports and many diets, both fad and medically-approved, exist to provide the optimum concentration of nutrition and calories to build strength, stamina and recovery. However, the culture and attitudes of the athletes within each sport will determine overall nutrition. In Japan, sports nutrition has been poorly studied among collegiate female students (Kawano et al., 1997; Nagasawa et al., 2004) and while there are also a few English-language reports on the impact of nutrition in athletes, they are not at the university level (Seiki 1992; Okano et al., 1993; Yamanaka et al., 1994; Okano et al., 1996). Furthermore, it has been reported in Japan (and other countries) that the actual level of support in sports nutrition is still poor and the awareness of the importance of nutrition intake among athletes and coaching staff are not always sufficient (Wiita et al., 1996; Kawai 2002; Iwami et al., 2005). Fortunately, as Japan's nationalized school system teaches Ministry of Health-approved guidelines on meal composition and frequency, we were able to assume that all survey participants were aware of "healthy" nutrition guidelines and that any deviation from them was a conscious choice brought about by either the culture of the sport/coaches or a personal decision based on athletic goals. In general, the intense sports (Group IIIA) were found to have lower frequencies of well-balanced meals while endurance athletes (Groups IIB and IIC) reported higher levels of well-balanced mealtimes. Additionally, Group IIIA athletes were found to have more frequently tried intentional weight loss diets. As Group IIIA sports are primarily short-twitch, energy burst-type sports that demand maximum strength-to-weight ratios, it could be that this kinesthetic pressure, in addition to pressure from peers and coaching staff, results in skipped or unbalanced meals (Baldari et al., 2001; Mcneal et al., 2007; Penitente et al., 2015). Likewise, the intensive need for long-twitch endurance and sustained energy release in Group IIB and IIC sports, coupled with long training sessions, drive those athletes towards well-balanced mealtimes (Sasaki et al., 2006). These results are in line with reports from other countries. In Australian female elite athletes, endurance sports participants (including distance runners and swimmers) showed a higher energy intake against weight-conscious groups (including gymnasts) reporting low energy intakes (Burke et al., 2003). In a study among elite Greek female aquatic athletes reported to have inadequate energy intake, the poor diets were considered to be healthy and well-balanced (Farajian et al., 2004). Furthermore, athletes in non-lean sports tend to be healthier and have fewer eating problems than did non-athletes (Smolak et al., 2000). However, it has been also reported that race / ethnicity are related to eating disorder classifications (Greenleaf et al., 2009) as ethnic/cultural perceptions of ideal body shapes and desire for thinness may vary highly (Wildes et al., 2001).

The trend of potential malnutrition in Group IIIA athletes is troubling, as skeletal, neurological, endocrine, reproductive and muscular development could impact Group IIIA athletes at a much greater frequency than the general population. Based on this data, coaching staff are recommended to retrain and focus Group IIIA athletes on the fundamentals of nutrition in an accountable manner.

4-4-1. The effects of competitive level on FAT factor risks

After finding that intensity/training volume effects on FAT factor risk were in line with reports from other countries, it was next sought to establish previously unreported links between competitive levels and menarche age/amenorrhea, musculoskeletal injury, and nutrition choices. A study in Japan reported that the rate of amenorrhea was 6% in female top competing level athletes and was higher than in non-athletes (Scientific reports from Japan Institute of Sports Sciences). Other studies in Japanese female top competing level athletes reported that 59.3% athletes had regular menstrual cycles while 32.9% of athletes had menstrual dysfunction (including 7.8% athletes with secondary amenorrhea) (Nose et al., 2014). In this study, athletes with irregular menstrual status were up to nearly 30% and athletes with secondary-amenorrhea were 5.6%, which was quite similar to these previous reports. As our own data were similarly reflective of this phenomenon, comparisons based solely on competitive level were made. To avoid bias that would come from simply evaluating groups, comparisons within each sport (LTH), each sport to its own group (STG) and each group to other groups (GTG) were also used. In this way, it was hoped to establish a baseline of identification for sport types and groups in which competitive influence on FAT exists.

With regard to menarche and competitive level, an overall trend of higher competitive level resulting in higher menarche ages though this effect disappeared in the group comparisons and sports type comparison were found. It is believed that this is due to sport intensity and the aforementioned hormonal imbalances associated with intense/high volume training being the key factors in delayed menarche (Merzenich et al., 1993). Competitive level, in the face of intense training and possible nutritive deficiencies, may simply be a complicating secondary factor.

However, with respect to competitive levels and amenorrhea, we found that the lower levels within groups had more menstrual irregularities as opposed to their highly competitive counterparts (with the exception of IC, where high competition correlated to more menstrual irregularities). This unusual finding is suggested to be due to a “veteran effect”, where higher levels of competition effectively filter out athletes whose bodies experience amenorrhea; these unadaptable athletes would be then forced to compete either at low levels or quit entirely. In Group IC, which includes endurance sports such as running and race walking, the effect of constant body fat mobilization may induce hormonal cascades that adversely affect menstruation at the higher competitive levels.

Next, the effect of competitive levels on musculoskeletal injuries were looked at and no general correlation were found between them. However, in Group IIC, the lower competitive levels had higher injury numbers (but not stress fractures) and in Group IIB, the preponderance of injuries suffered were stress fractures. As field events like jumping and sprinting cause microfractures that may worsen due to the repetitive nature of those sports, it has been reported that these athletes are at a higher risk of stress fractures (Mayer et al., 2014). Group IIC sports included team sports such as basketball and lacrosse as well as swimming and rescue swimming. At the lower level in these sports, higher injuries might be due to the relative inexperience of the athletes but the fact that the stress fracture is less common than other types of injuries could be explained by either the lack of impact (*e.g.*, swimming) or the constant, low-level motion required to follow the ball (basketball, lacrosse) or stay with the pack (mid-distance running).

As nutrition and body fat percentage are thought to be key factors in developing FAT (Nattiv et al., 2007), the possible relationships between competitive level and nutrition choices were analyzed. Within the groups, only IIC showed a correlation between competing level and positive nutrition choices. Within the individual sports, lacrosse players were most likely to often eat well-balanced meals. This could be explained by the need for a large store of energy to draw from while running at medium distances. Lacrosse and basketball sports in particular have a stop-and-go model where high intensity bursts are interspersed into long bouts of lower level physical motion; research has shown these athletes have a high requirement for easily utilized carbohydrate energy (Williams et al., 2015).

Interestingly, competitive levels did affect variables such as starting ages of sports and the average heights of the athletes in them. Higher starting ages were seen in higher levels of competition in Groups IB and IIIA sports. Within these groups, Group IIIA sports such as artistic gymnastics had a much higher starting age than other sports such as volleyball or basketball. The musculoskeletal demands of gymnastics may require a higher starting age as coordination and motor skills are selective pressures in this sport (di Cagno et al., 2014). As for height, fencing, volleyball and throwing naturally require either longer reach (fencing), ability to reach over the net (volleyball), or body height/weight as leverage (throwing) but the scoring demands of Group IIIA sports may mean that, within the sport itself, the competitive pressure selects for taller and more “graceful” athletes (Georgopoulos et al., 2001). Interestingly, basketball, which would be expected to have taller athletes, was not enough to lift Group IIC into significance when compared to other groups. However, as basketball is a team sport, shorter athletes who have power and skill may be able to compensate for height disadvantage and not every position on the team requires exceptional height in women's basketball. In fact, Japanese female athletes in general would not be expected to be more than 169cm at maturity (only 0.3% of the population would exceed this mark), thereby selecting more for power and skill than pure height (Thomas et al., 1990).

Several limitations must be acknowledged. First, no actual diagnoses of FAT risk factors were clinically conducted; relationships to risk factors were calculated based solely on survey responses. Second, surveys were not collected at the immediate end of each season and medical records for each of the athletes were not consulted to verify answers. Overreliance on the recollections and self-reports of athletes may have introduced errors into the data. Measurement of menstrual cycles or monitor estrogen levels also have not been done within athletes during the study, relying solely on questionnaires, which may not capture a complete picture of injury risk during the various phases of the cycle. Finally, comparisons that isolate relationships between variables may conceal multivariable relationships. However, to the best of the knowledge in this study, this is the first report of its kind with a large sample size that examines the parameters of Japanese female collegiate athletes with respect to FAT and will serve as a foundational report upon which to build future prospective studies featuring clinical validation of FAT risk factors.

As a summary to this study, Japanese collegiate athletes were found to experience the FAT risk factors (delayed menarche/amenorrhea, musculoskeletal injury, and poor nutrition) in a manner correlated with the intensity of their sport types and, to a lesser extent, the volume of training endured. Of particular note is the trend of repetitive motion injury in endurance/non-dynamic sports. Additionally, some correlation between lower competitive levels and delayed menarche/amenorrhea were found which have been considered the effect of chronically low ovarian hormone level. However, in general, competitive level had no effect on musculoskeletal injury or nutrition choice. Taken together, results in this study highlight the need for coaching staff and universities to adjust sport rules and expectations to compensate for the higher risks of FAT in athletes subjected to high intensity, high training volume and highly competitive sports.

5. Chronically low estrogen status increases muscle soreness, muscle damage biomarker levels and tendon stiffness after heavy exercise in Japanese collegiate athletes

5-1. Introduction

Intensive training schedules coupled with the unique characteristics of female physiology has resulted in more location-specific injuries, such as in the back/neck and lower body (Sallis et al., 2001), even though, in general, women are less prone to fatigue than men (Avin et al., 2010). Female athletic performance in areas such as static balance ability (Hayashi et al., 2004), jumping ability (Hayashi et al., 2004), and agility (Rashmi et al., 2017) increase when estrogen is high and, in addition, estradiol has been previously reported to play an important role in membrane stabilization (Claassen et al., 2005) and antioxidant status (Enns et al., 2010). It suppresses the elevation of muscle damage marker creatine kinase (CK) and histological muscle damage after exercise (Amelink et al., 1988; Bär et al., 1988; Komulainen et al., 1999: Tiidus et al., 2001). Studies in men (effectively low estrogen) have reported that basal CK levels are significantly higher, bolstering this line of thinking (Mougois 2007). However, the cycling of hormones may introduce variability into performance and recovery that could increase musculoskeletal injury risk as a growing body of evidence links the various phases of menstruation to athletic performance. Julian and colleagues found that maximal endurance dropped at the mid-luteal phase in sub-elite soccer athletes while Pallavi and colleagues recently found a correlation between decreased grip strength and phases 1 and 3 of menstruation (Julian et al., 2017; Pallavi et al., 2017). Numerous other studies have also probed the relationship between the menstrual cycle (including related symptoms) and athletic performance, including increases in injury risk (Jullian et al., 2017; Martin et al., 2017; Konopka et al., 2016; Lee et al., 2018). Furthermore, the linking menstrual cycling and edema was shown in Chapter 3. Therefore, maintenance of regular cycles seems to be critical to maintaining athletic performance. However, it has been reported that approximately 80% of top-level female athletes and 50% of regular female athletes suffer from menstrually-related symptoms (Kaimura et al., 2014; Nakamura et al., 2012; Takeda et al .,2015) while another study found that approximately 40% of high-competition level Japanese athletes were reported to have menstrual dysfunction (mainly primary amenorrhea), secondary amenorrhea, polymenorrhea, and irregular cycles (Nose et al., 2014). Furthermore, menstrual irregularity including secondary amenorrhea in athletes were linked with their low competing level which were shown in chapter 4.

Prolonged suppression of ovarian hormone levels (such as found in amenorrhea) seems particularly indicative of higher injury risk (especially bone stress fractures) (Nose et al., 2014). Thus, hormonal status is clearly an issue of concern in sports medicine with respect to the musculoskeletal health of the participants.

Therefore, the aim of this study was to investigate the differences in muscle damage after acute, heavy intensity exercise in Japanese ovary-suppressed athletes and eumenorrheic athletes. It was hypothesized as due to chronic hormonal imbalance, athletes in an ovary-suppressed state would show a higher subjective muscle soreness, higher basal CK and lactose dehydrogenase (LD) levels, and significant elevation in tendon stiffness after exercise.

5-2. Methods

5-2-1. Subjects

Nineteen female collegiate athletes (ages 18-22) at the University of Tsukuba, Tsukuba City, Japan were studied from July 2017 to March 2018. Inclusion criteria were: physically healthy, non-smokers, familiar to resistance training, and not taking medications (including oral contraception). All participants received an explanation of the purpose and flow of the study and signed an informed consent form prior to their inclusion. All aspects of the study were approved by the Ethics Committee of the University of Tsukuba (Approval # 29-19).

5-2-3. Study Procedures

5-2-3-1. Group categorization

Starting at least 1 month prior to the measurement, the participants recorded their basal body temperatures every morning upon awakening. Normal menstrual cycles and ovulation were confirmed by a gynecologist based on basal body temperature data analysis and used to retrospectively divide athletes into 2 groups according to ovarian status at baseline: either the cyclic menstrual function (CYC) group or the menstrual dysfunction (DYS) group (Vanheest et al., 2014). Athletes who had cyclic menstruation 1 year prior to evaluation started their first measurement at the nearest day without club activity at least 3 days post-menstruation. Athletes who reported irregular menstrual cycles (less than 5 times over the prior year) started on any convenient day. Because all athletes had at least one particular day of the week without club activities, the first measurement in each condition were considered as the week 1 timepoint, the second measurement as the week 2 timepoint, and the third measurement as the week 3 timepoint.

5-2-3-2. Rest vs. Exercise Test Conditions

All measurements were done on days with no sports activities and participants were asked to abstain from athletics 12 hours before evaluation. Measurements were taken under 2 conditions: rest (RE) and exercise (EX) over the 3-week period. During RE, athletes sat quietly for 60 minutes. For the exercise protocol, athletes performed an acute heavy resistance exercise test consisting of 6 sets of 5 parallel squats at 90% of their previously determined one-repetition maximum weight (1-RM) with a 3-minute rest between each set (Wolf et al., 2012).

5-2-4. Preliminary measurements

The actual parallel squat 1-RM test were performed in each participant prior to the study. All participants did the 1-RM test just once. When participants were able to perform more than one repetition, an additional set with a heavier load was performed in order to reach the 1-RM. Sets were separated by 5 min of passive rest. All participants were experts on the parallel squat. Throughout the preliminary and main trials, participants were supervised by at least two investigators.

5-2-5. Bloodwork and physiological testing

Body composition, blood biochemistry (including ovarian hormones), serum CK, LD, blood pressure and heart rate, stiffnesses of biceps femoris tendon and semimembranosus / semitendinosus tendon, and muscle soreness were measured before (pre), immediately after (post 0), 30 minutes after (post 30), 60 minutes after (post 60), and 24 hours (post 24H) after both conditions.

5-2-6. Measurements

Basal body temperature was measured orally using digital thermometers (CTEB503L, Citizen Co, Ltd., Tokyo, Japan). Body composition analyzer (BC-118E, Tanita Co., Ltd., Tokyo, Japan) were used to obtain anthropometric data consisting of weight, body fat mass, percent body fat, and body water volume. Serum estradiol and serum progesterone concentrations were measured using a Chemiluminescence Enzyme Immunoassay (CLEIA) and CK/LD levels were analyzed by an independent laboratory (LSI Medience, Ibaraki, Japan). Systolic/diastolic blood pressure and heart rate were evaluated by an automated measurement system (HBP-1300, Omron Co., Ltd., Kyoto, Japan). Stiffnesses of biceps femoris tendons and semimembranosus / semitendinosus tendons were assessed using SOFTGRAM (Shinko Denshi, Co., Ltd., Japan). The equipment used in this study to evaluate the tendon stiffness is based on the previous reports (Tani et al., 2009; Tani et al., 2010), which is considered to be more potable and less invasive than ultrasound and muscle hardness meter. The tendon stiffness was evaluated by a skilled athletic trainer. Athletes were instructed to be on a face-down position with their knee joint slightly bent. After that, they were asked to resist while the athletic trainer tries to push the leg to the extension direction, and detected the biceps femoris tendons and semimembranosus / semitendinosus tendons near the knee. The measurement was done 3 times respectively, and the median was used for the analysis. Muscle soreness was determined by marking a 100mm visual analog scale (VAS) bordered by the terms “no pain” and “the worst pain ever experienced.” Athletes rated their perception of soreness on rising from a seated position by using a sticker to make a mark on the line to indicate their feeling of muscle pain relative to those two extreme points (Oosthuyse et al., 2017). The mark was then measured as the distance from the left border of no pain. The use of a VAS evaluate pain has been previously validated (Ferreira-Valente et al., 2011).

5-2-7. Statistical analysis

All data are presented as means ± SDs. SPSS version 25.0 (SPSS Inc, Chicago, IL, USA) were used to analyze in group × time using repeated measure ANOVA. Bonferroni's post hoc test was performed only if F achieved statistical significance ($p<0.05$) and there was no significant variance inhomogeneity. One-way ANOVA was used to analyze the significance in each group (CYC, DYS) in each condition (RE, EX) over the time points (0, 30 min, 60 min, 24H). It was also used to compare each of the 3 weeks with respect to the same conditions/groups and a Bonferroni post hoc test was conducted when a significant main effect was shown. Additionally, non-paired t-testing was performed to evaluate the difference between 2 groups at each measurement point, for each condition, within the level of ovarian hormones for each week, and within the information of age, height, age of menarche, and menstrual cycle between the groups. The sample size calculations yielded a minimum total sample size of 18 to attain a statistical power of 0.50 in the measurements. $P \leqq 0.05$ was considered as statistically significant.

5-3. Results

5-3-1. Anthropometric parameters related to menstruation did not significantly differ between ovary-suppressed and eumenorrheic athletes under rested or exercised conditions

Neither significant interactions nor significant changes were seen in each group, each week, or each condition with respect to body weight, body fat mass, percent body fat, or body water volume. With the exception of the EX condition significantly increasing systolic blood pressure and heart rate at post 0, there were also no significant interactions in blood pressure and heart rate for all time points and conditions.

5-3-2. Menstrual cycle length is higher in the DYS group and ovarian hormone levels are significantly higher in rested and post-exercise conditions within eumenorrheic athletes

Table 5.1 shows the descriptive characteristics and differences between CYC (n=9) and DYS (n=10). There were no significant differences in age, height and the age of menarche; however, menstrual cycle length during the study was significantly higher in DYS (31.0±1.0 vs. 78.5±13.0 days, $p<0.01$), including 2 amenorrheic athletes who reported a menstruation delay of more than 90 days.

Table 5.1 Basic Characteristics

Basic characteristics	DYS group (n=10)	CYC group (n=9)	p value
Age (years)	19.8 ± 1.0	20.7 ± 1.0	0.081
Height (cm)	163.8 ± 7.4	161.7 ± 4.4	0.468
Weight (kg)	57.2 ± 8.5	58.3 ± 10.5	0.802
Body fat mass (kg)	21.4 ± 4.4	22.0 ± 2.6	0.714
Percent body fat (%)	12.4 ± 4.0	12.8 ± 3.5	0.826
Body water volume (kg)	32.8 ± 3.7	32.7 ± 3.5	0.663
Age of menarche (years)	13.8 ± 1.3	13.4 ± 13.4	0.538
Menstrual cycle (days)	78.5 ± 13.0 *	31.0 ± 1.9	<0.01

The data represent mean ± SD, * $P<0.05$ between DYS group and CYC group

Ovarian hormone concentrations in each groups and weeks are shown in Figure 5.1. There were no significant differences between serum estradiol and progesterone levels within the DYS group over the 3-week period in the RE. Estradiol levels in the CYC group significantly increased in week 2 in the EX condition compared to week 1 (p=0.015), while week 2 and week 3 estradiol significantly increased compared to week 1 in the RE condition (p=0.02 and 0.022, respectively). Progesterone levels in the CYC group significantly increased in week 3 compared to week 1 in the EX condition (p=0.009), while week 3 was significantly higher than week 1 and week 2 in the RE condition (p=0.021 and 0.023, respectively).

In group comparisons, estradiol levels were significantly higher in the CYC group compared to the DYS group in week 2 and week 3 under both RE and EX conditions. Additionally, progesterone levels were significantly higher in week 3 in the CYC group versus the DYS group under both conditions.

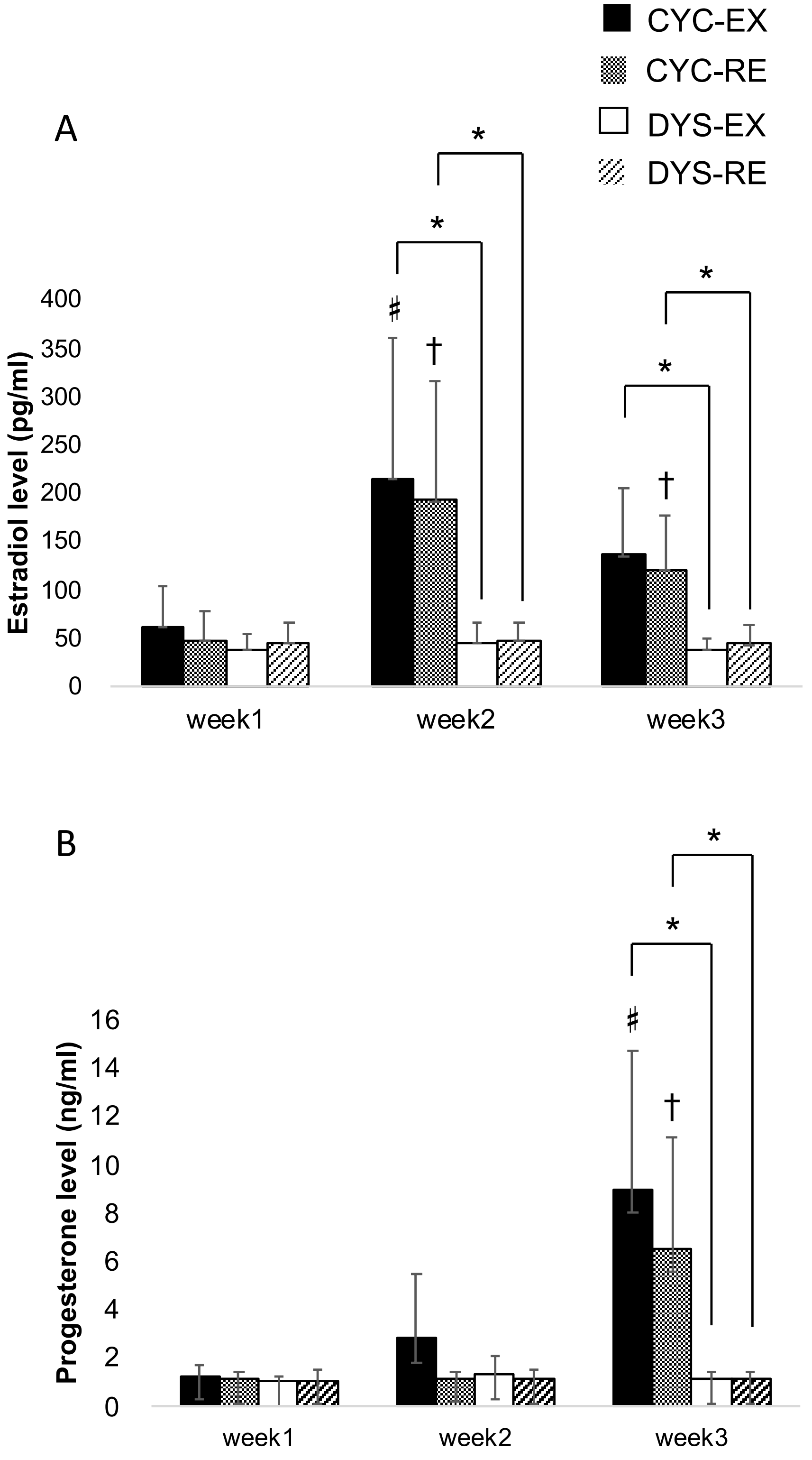


Figure 5.1 Ovarian hormone concentrations

Ovarian hormones in CYC group are shown in black square and shade square for EX and RE condition, respectively. DYS group are shown in black and stripe square for EX and RE condition, respectively.

♯ shows $P<0.05$ vs. week 1 in EX condition and † shows $P<0.05$ vs. pre in RE condition. * shows $P<0.05$ between CYC and DYS group in each condition.

5-3-3. Muscle damage markers are increased at all time points in the ovary-suppressed state compared to the eumenorrheic state

The CK and LD levels in each group for both conditions are shown in Figure 5.2 and Figure 5.3, respectively. There were no significant interactions between CK and LD levels for both RE and EX conditions. Additionally, both the CYC and DYS groups in the RE condition showed no significant differences in CK and LD at any time point. In CYC, the CK level in EX condition significantly increased at each post-exercise time point compared to pre in week 1 and 2, and significantly increased at post 60 and post 24H compared to pre in week 3. Additionally, post 24H was significantly higher than post 0 in all weeks, but significantly higher than post 30 only in week 3. CK after exercise in the DYS group was significantly higher only in post 60 and post 24H in week 1, post 24H in week 2, and significantly higher in post 24H compared to all other points in week 3. However, CK levels in DYS were significantly higher than CYC in all time points over all weeks.

Over all weeks, LD levels in the CYC group increased significantly only in post 30 and post 24H. LD levels in DYS on the other hand, increased significantly from pre in all time points over all weeks. Comparatively, DYS was significantly higher than CYC at every time point in weeks 2 and 3, but only in post 30 in week 1.

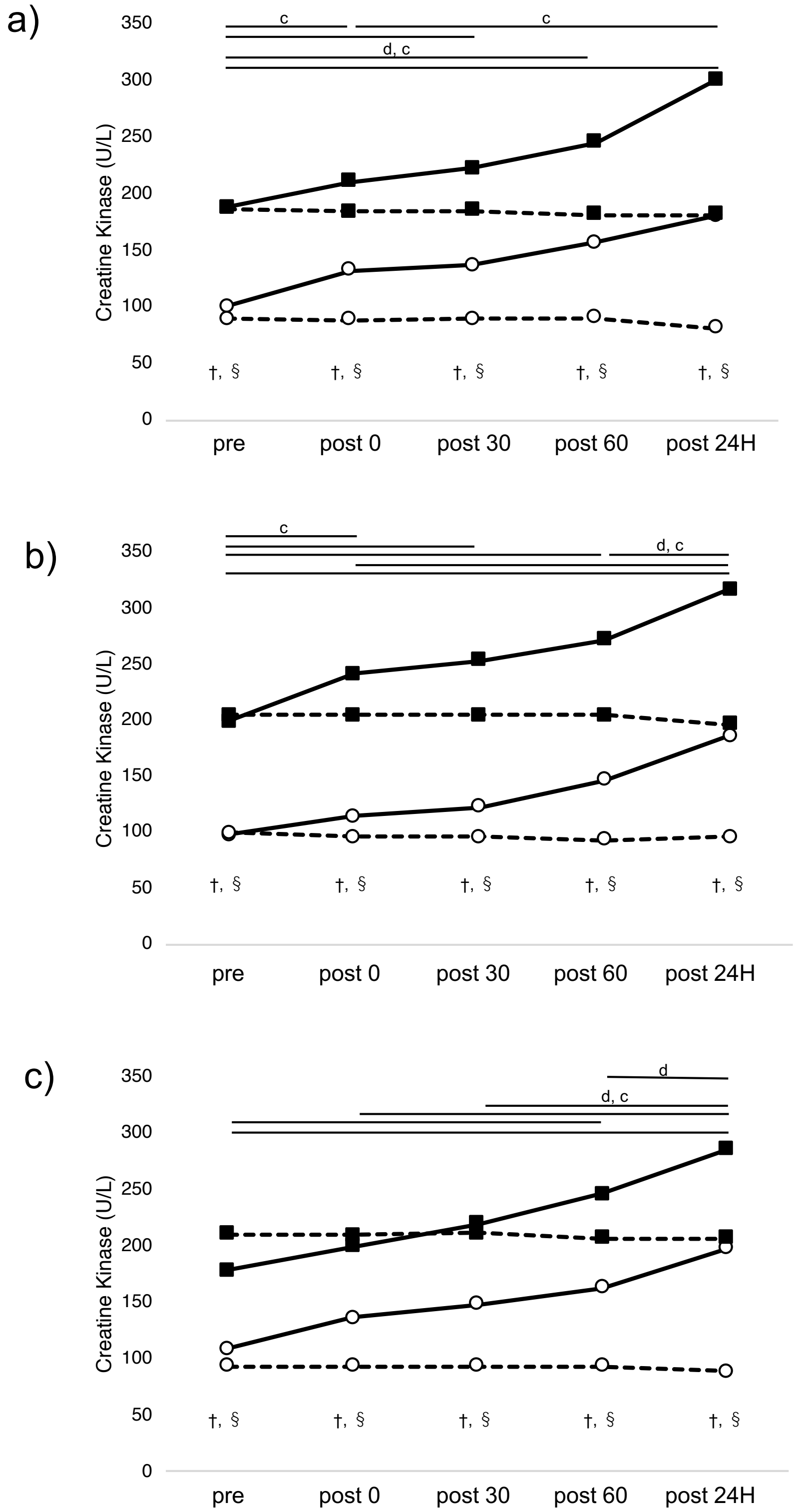


Figure 5.2 Creatine kinase variation in exercise and rest conditions in each week

a), b) and c) shows the variation of creatine kinase level in week 1, week 2 and week 3, respectively. DYS group in EX and RE conditions are shown in solid and dashed lines, respectively, with black squares. The CYC group in EX and RE conditions is shown in solid and dashed lines, respectively, with white circles. d and c are shown as $p<0.05$ between time points in DYS and CYC groups, with respect to the EX condition. † are shown as $p<0.05$ between groups in the EX condition and § are shown as $p<0.05$ between groups in the RE condition.

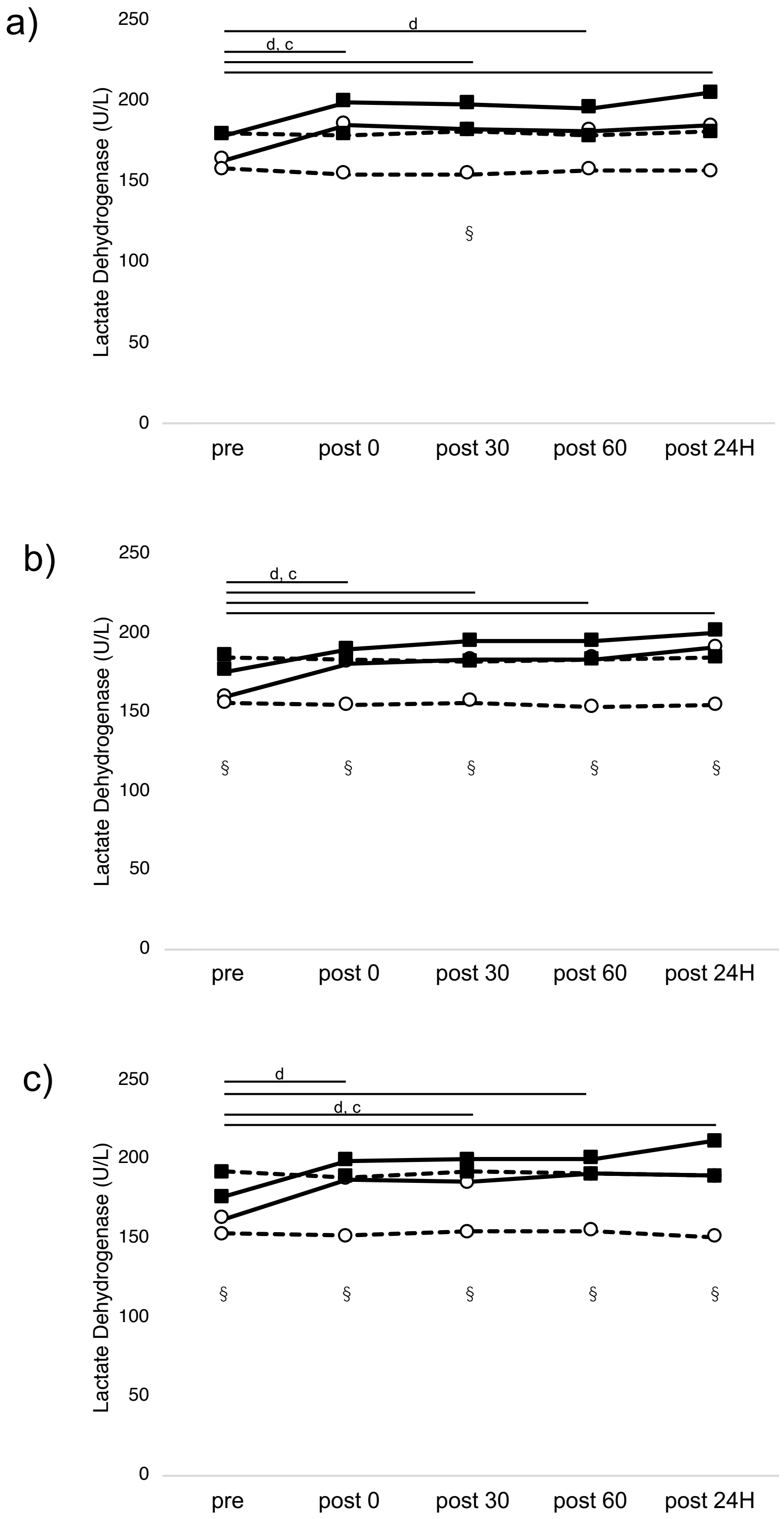


Figure 5.3 Lactate dehydrogenase variation in exercise and rest conditions in each week

a), b) and c) shows the variation of Lactate Dehydrogenase level in week 1, week 2 and week 3, respectively. DYS group in EX and RE condition are shown in solid and dashed lines, respectively, with black squares. The CYC group in EX and RE conditions are shown in solid and dashed lines, respectively, with white circles. d and c are shown as $p<0.05$ between times points in DYS and CYC group, with respect to the EX condition. § are shown as $p<0.05$ between groups in the RE condition.

5-3-4. The ovary-suppressed state increases biceps femoris tendon stiffness 24 hours after exercise versus the eumenorrheic state

There were no significant interactions in stiffness between the biceps femoris tendon and semimembranosus / semitendinosus tendon under RE and EX conditions. Both CYC and DYS in the RE condition, and CYC in EX condition showed no significant stiffness differences at any time point. However, while there was no significant difference in semimembranosus / semitendinosus tendon stiffness within the EX condition in DYS, the stiffness of the biceps femoris tendon significantly increased in post 24H in all weeks (Figure 5.4).

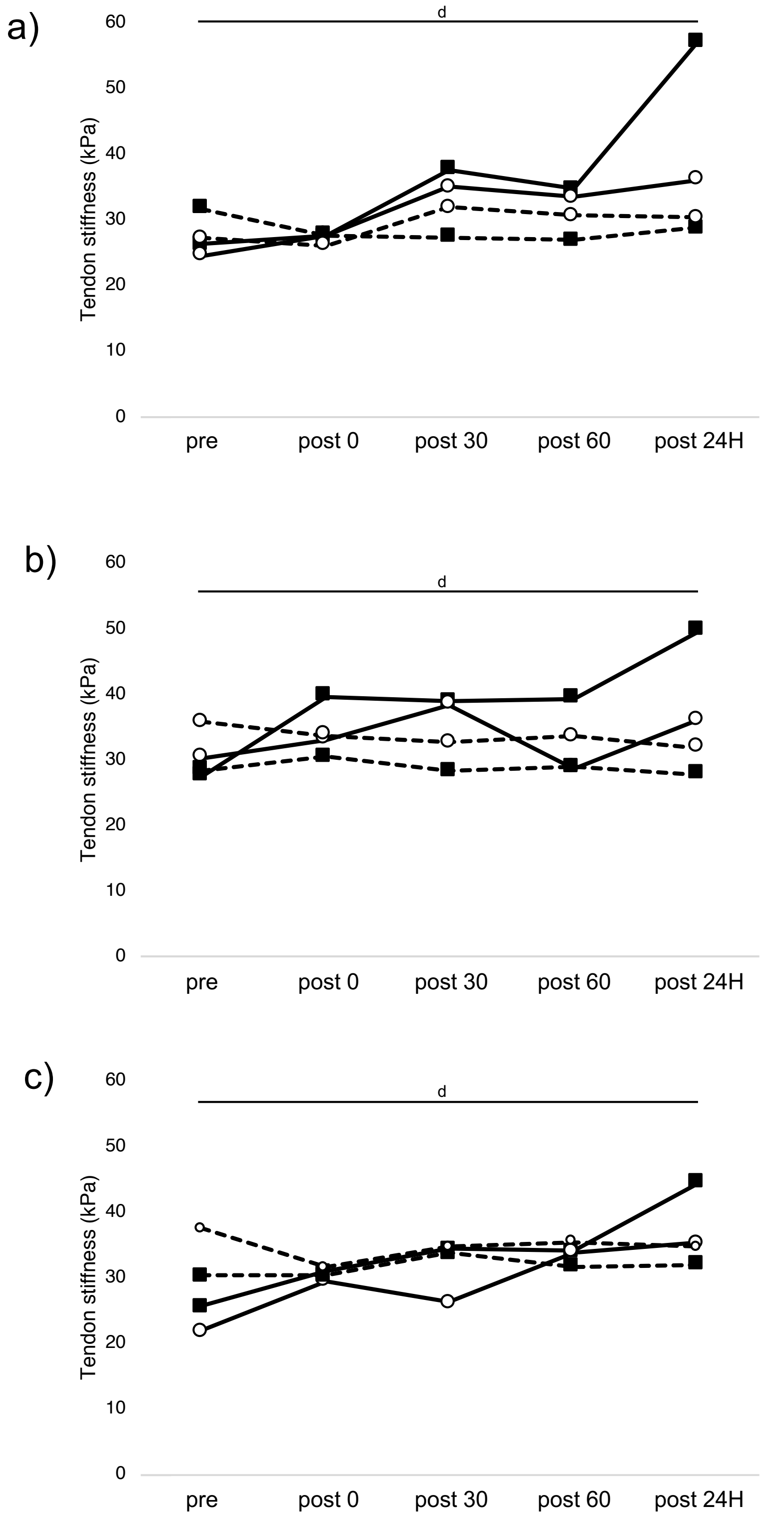


Figure 5.4 Variation of tendon stiffness of femoris biceps in each week in exercise and rest conditions in each week

a), b) and c) shows the variation of tendon stiffness in week 1, week 2 and week 3, respectively. The DYS group in EX and RE conditions is shown in solid and dashed lines, respectively, with black squares. The CYC group in EX and RE conditions is shown in solid and dashed lines, respectively, with white circles. d are shown as $p<0.05$ between times points in the DYS group in EX condition.

5-3-5. Ovary-suppressed conditions increase short-term subjective muscle soreness but do not affect muscular adaptation to intense exercise

There were no significant interactions nor differences over the time points for both DYS and CYC with respect to RE condition and muscle soreness. In the CYC group, the EX condition saw a significant soreness increase in post 0 in week 1 and 2, but this significantly decreased at post 24H compared to post 0 in week 3 (adaptation to exercise). Muscle soreness of DYS in EX vs RE condition significantly increased in post 0, post 30, post 60, post 24H in week 1 and 2, and increased significantly only in post 0 compared to pre in week 3 (Figure 5.5).

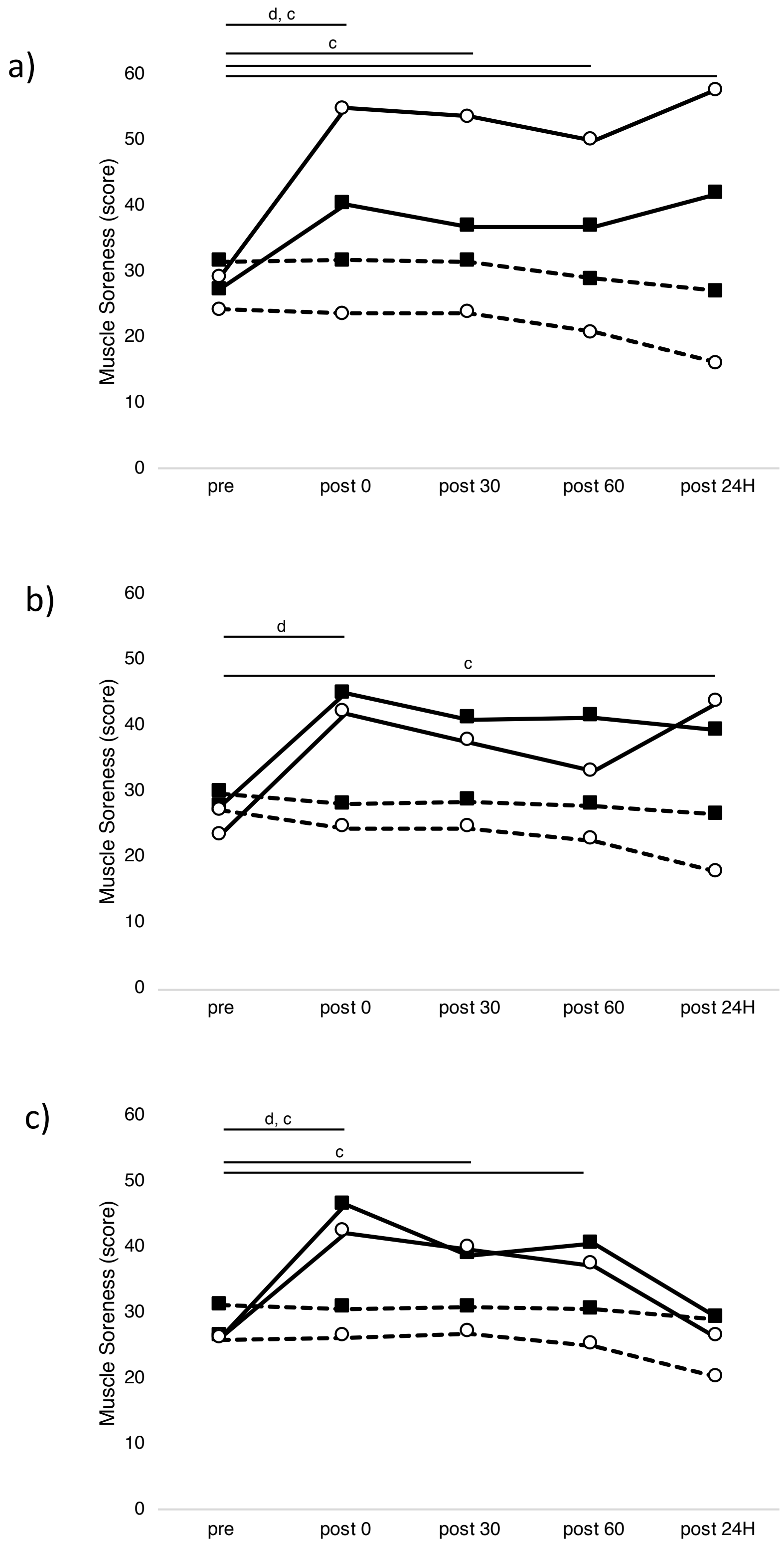


Figure 5.5 Muscle soreness score in each week in exercise and rest conditions in each week

a), b) and c) shows the variation of muscle soreness score in week 1, week 2 and week 3, respectively. The DYS group in EX and RE conditions is shown in solid and dashed lines, respectively, with black squares. The CYC group in EX and RE condition are shown in solid and dashed lines, respectively, with white circles. d and c are shown as $p<0.05$ between times points in DYS and CYC group, with respect to the EX condition. † are shown as $p<0.05$ between groups in the EX condition.

5-4. Discussion

5-4-1. Anthropometric characteristics related to menstrual status

Low body weight, and low body fat volume associated with amenorrhea or oligomenorrhea were not seen in this study. The homogeneity of the participants and the exclusion of advanced symptoms of amenorrhea (i.e., weight loss or anemia) would therefore make this data more indicative of trends associated with the larger population of relatively healthy Japanese female college athletes.

5-4-2. Ovarian hormone levels and cycle length

Only the CYC group showed variance in ovarian hormonal levels. This is in line with the knowledge that the menstrual phase is marked by low ovarian hormone concentration which elevates as the ovulatory phase is entered. According to a previous report (Jordan et al., 1994), serum progesterone concentrations of at least 5 ng/ml are indicative of adequate luteinization which is in line with the CYC group results. Meanwhile, DYS athletes showed low levels of both ovarian hormones during all weeks in both conditions. During measurement, 2 athletes had not menstruated for more than 3 months (amenorrhea) while other 8 athletes had oligomenorrhea. However, while all DYS athletes reported to have had a menstrual cycle 1 year prior to the study (albeit less than 5 cycles per year), heavy training seemed to result in frequent cycle delays which is in line with previous reports on amenorrhea (Baxter-Jones et al., 1994; Dušek 2001 and 2004; Redman et al., 2005).

5-4-3. Muscle soreness

Although muscle soreness was significantly higher in CYC than in DYS in post 30 of the EX condition in week 1, the magnitude of muscle soreness from pre-exercise was significantly higher in post 0 in all weeks in DYS. In previous reports focusing on sex differences, muscle soreness peaked at 24 hours after downhill exercise in both men and women in follicular phase though the soreness in women did not recover to the pre-exercise level 72 hours after exercise while men did (Oosthuyse et al., 2017). However, another study saw no significant sex differences in muscle soreness 4, 7, and 10 days after exercise (Sewright et al., 2008). Over the menstrual cycle, it has been reported that the recovery of muscle soreness was faster in mid-luteal phase than in early follicular phase and late follicular phase, which would explain the lower soreness in the eumenorrheic group (Oosthuyse et al., 2017). However, other conditions, such as water retention and general malaise or tiredness that may accompany normal menstruation, may force athletes to train less hard and effect less muscle soreness in these high-hormonal phases.

5-4-4. Muscle damage markers

In previous reports on post-exercise CK levels during the menstrual cycle, a negative correlation was seen between CK level variance before and after downhill running and estrogen level in mid-follicular phase (high estrogen, low progesterone) (Carter et al., 2001). In another report, CK level significantly increased 24 hours after downhill running in early-follicular phase (EF), late-follicular phase (LF), and mid-luteal phase (ML) though no significant differences between the phases were observed (Oosthuyse et al., 2017). Although they were not correlated, both CK and LD were found to be significantly higher in DYS than CYC at all time points similar to one report showing a higher basal CK level in amenorrheic athletes than eumenorrheic athletes (Thompson et al., 2006). Attempts have been made to characterize human sex differences in CK response after resistance exercise, reporting greater levels in males than in females (Wolf et al., 2012) as evidence of the protective effect of estrogen. Hence, it is necessary to fully investigate how menstrual status affects exercise recovery in a mechanistic fashion to reinforce the suggestion that DYS status delays recovery due to estrogen fluctuation.

5-4-5. Tendon stiffness

Significant stiffness increases after 24 hours of exercise for all weeks in the DYS group were seen only in the biceps femoris tendon. Ligament stiffness (especially in the anterior cruciate ligament) is reported to decrease in ovulatory phase (high estrogen level) (Eling et al., 2007) but no variances related to menstruation have also been reported (Kubo et al., 2009; Burgess et al., 2009; Burgess et al., 2010). As estrogen receptors are known to be present in tendon/ligament fibroblasts (Kjaer and Hansen 2008), hormone-related differences were expected in spite of these conflicting reports.

In contrast to the ovary-suppressed state, there was no significant change in tendon stiffness through the hormone fluctuation in the eumenorrheic group over the 3-week period of this study. This may seem counterintuitive when based solely on the theory of estrogen relating directly to stiffness, but it can be suggested that short-term fluctuations in normal menstruation do not affect tendons while chronically imbalanced hormones have a more significant impact on stiffness. Furthermore, edema, which is controlled by various factors in addition to menstrual status, may also contribute to tendon stiffness which was seen in Chapter 3.

As a summary, it is suggested that the delay of menstruation due to chronically low ovarian hormone concentrations may lead to higher muscle damage (both in CK levels and soreness) as well as increased tendon stiffness after exercise. Furthermore, these data indicate a deeper relationship between chronic menstrual irregularities that cannot be explained by simple estrogen/estradiol levels. Further investigations are therefore needed to determine whether hormone replacement therapy or other biochemical interventions could be useful to combat the impact of amenorrhea on musculoskeletal performance and recovery.

6. Muscle damage response in ovary-suppressed athletes after a highly intensive 4-day training camp

6-1. Introduction

Increased participation in sports by women has led to enhanced interest in the physiological and metabolic responses of women to sport and exercise (Constantini et al., 2005). In Chapter 3, menstrually related edema showed a negative correlation with agility, suggesting a decline in one aspect of athletic performance which correlated to ovarian hormone levels. Additionally, Chapter 4 showed that athletes with irregular menstrual statuses were significantly more numerous in low competing levels, suggesting that menstrual irregularity, including chronically low ovarian hormone levels (*e.g.,* amenorrhea), is directly or indirectly related to competitive performance. The literature supports this argument as it has been reported that ovary-suppressed athletes with chronically low ovarian hormone levels showed less training adaptation than regularly cycling athletes (Vanheest et al., 2014).

Estradiol has been previously reported to play an important role in membrane stabilization (Claassen et al., 2005) and antioxidant activity (Enns et al., 2010) *in vivo* and *in vitro*. Additionally, estrogen is reported to suppress the elevation of creatine kinase (CK) levels and histologically visible muscle damage after exercise in animals, enhancing the idea of a sex difference (Tiidus et al., 2001; Komulainen et al., 1999; Tiidus et al., 2001). Studies in men have shown basal CK levels are significantly higher in males (who are basically under constant low estrogen levels) than females (Mougois 2007). Although one previous study has reported greater CK activity in men than women 4, 7 and 10 days after an eccentric resistance exercise (Sewright et al., 2008), this result is impractical for athletes who are incapable of such long-term rest periods. Resting serum CK levels have previously been reported to be higher in amenorrheic athletes who persistently have low estrogen levels, than in eumenorrheic athletes (Thompson et al., 2006). However, this study did not evaluate the post-exercise CK response. Studies in postmenopausal women have reported higher CK levels after eccentric exercise in women who do not take hormone replacement therapy than in women who do (Dieli-Conwright et al., 2009). Similarly, CK levels in a eumenorrheic late follicular phase (in which only estrogen rapidly increases) significantly decreased 24 hours after a downhill exercise compared to early follicular phase and mid-luteal phase (Oosthuyse et al., 2017). Studies focusing on muscle response after exercise have been reported previously, though none considered the menstrual phase or status in their studies (*i.e.,* eumenorrhea, amenorrhea, oral contraceptive use, postmenopausal, etc.) (Sorichter et al., 2001; Sewright et al., 2008), or measure in only one phase (Stupka et al., 2000; Minahan et al., 2015). The protective effect of estrogen against muscle damage following heavy exercise has been previously reported in many studies as mentioned above; however, the muscle damage response following heavy exercise in ovary-suppressed athletes (who are characterized with persistently low ovarian hormone levels) and any differences with eumenorrheic athletes has not been thoroughly explored. In Chapter 5, muscle damage after acute, heavy intensity exercise was greater in athletes with chronically low ovarian hormone levels (menstrual disturbances) than in athletes with regular menstrual function. In line with low ovarian hormone levels, the basal CK levels and muscle tendon stiffness 24 hours after exercise were significantly higher in athletes with prolonged low ovarian hormone levels than regularly cycling athletes.

Therefore, the aim of this specific study was to investigate the differences in muscle damage response after an intensive 4-day training camp in ovary-suppressed athletes and eumenorrheic athletes. The hypothesis to this specific study was that athletes with chronically low ovarian hormone levels will show a statistically significant delay in muscle damage after the training camp versus eumenorrheic athletes.

6-2. Methods

6-2-1. Subjects

28 female collegiate athletes belonging to the basketball team at the Japan Women's College of Physical Education, Tokyo, Japan were studied from July 2018 to September 2018. Inclusion criteria were: physically healthy, non-smokers, not taking medications (including oral contraception), and those who completed the 4-day training camp and all 5 time measurements. All participants received an explanation of the purpose and flow of the study and signed an informed consent form prior to their inclusion. All aspects of the study were approved by the ethics committees of both the University of Tsukuba (#29-84) and the Japan Women's

College of Physical Education (#2017-3-3).

6-2-2. Experimental design

6-2-2-1. Group categorization

Starting at least 1 month prior to the measurement, the participants recorded their basal body temperatures every morning upon awakening. Normal menstrual cycles and ovulation were confirmed by a gynecologist based on basal body temperature data analysis and used to retrospectively divide athletes into 2 groups according to ovarian status at baseline: either the cyclic menstrual function (CYC) group or the Menstrual dysfunction (DYS) group according to a previous report (VanHeest et al., 2014).

6-2-2-2. Time points of measurements

Time points in this study are shown in Figure 6.1.

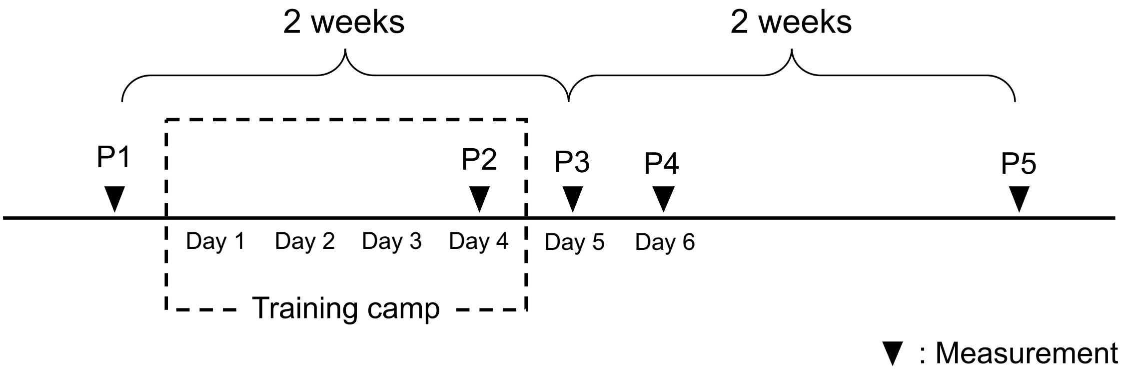


Figure 6.1 Study procedure

The day prior to P1, P4, and P5 was the day participants had no club activity. During the study, all participants completed the same training course (including the training camp). The training course during the camp is shown in Table 6.1. During the training camp, there were two training sessions per day from roughly 09:00 to 12:00 and from 14:00 to 17:00, except Day 1 and Day 4. The training in Day 1 was from roughly 14:00-17:00 and Day 4 was from 9:00 to 12:00. Warming up mainly consisted of dynamic movements and stretching. Technical training included short and repetitive basketball-specific movements, such as rebound and coordination training. During team training, game style movements with mainly 4-on-4 formations were done. The fundamental training was for improving basic physical fitness, including agility, power, and speed. The running training included short to middle distance interval training. Furthermore, all participants had the same meals during the camp.

Table 6.1 Training menu through the 4-days training camp

Day 1		Day 2		Day 3		Day 4	
AM	PM (14:30-17:00)	AM (9:00- 11:30)	PM (14:00- 17:30)	AM (9:00- 11:30)	PM (14:00- 17:00)	AM (9:00-)	PM
Move	Warm up (10 min)	Warm up (15 min)	Warm up (20 min)	Warm up (20 min)	Warm up (15 min)	Warm up (15 min)	Move
	Fundamental training (30 min)	Technical training (70 min)	Technical training (35 min)	Technical training (60 min)	Technical training (20 min)	Fundamental training (15 min)	
	Technical training (85 min)	Team training (40 min)	Team training (80 min)	Team training (50 min)	Team training (100 min)	Technical training (40 min)	
	Team training (30 min)		Fundamental training (20 min)		Fundamental training (35 min)	Team training (25 min)	
	Fundamental training (20 min)						

6-2-2-3. Measurements

Body composition, fasting blood biochemistry (including ovarian hormones), serum CK, LD, blood pressure and heart rate, stiffnesses of biceps femoris muscle and gastrocnemius, and muscle soreness were measured at all points from 7:00 – 9:00 a.m. Basal body temperature was measured orally using digital thermometers (CTEB503L, Citizen Co, Ltd., Tokyo, Japan). A body composition analyzer (ITO-Inbody370, Ito Co., Ltd., Saitama, Japan) were used to obtain anthropometric data consisting of weight, body fat mass, and body water volume. Systolic/diastolic blood pressure and heart rate were evaluated by an automated measurement system (HEM-7511T, Omron Co., Ltd., Kyoto, Japan). Stiffnesses of biceps femoris muscle and gastrocnemius muscle were assessed using a digital muscle stiffness scale (NEUTONE, TDM-Z, TRY-ALL Co., Ltd., Chiba, Japan). TMD (Total mood of disturbance) scores were calculated using the shortened version of the Profile of Mood States (2nd edition), which is a summary score for emotional state subscales. Muscle soreness was determined by marking a 100mm visual analog scale (VAS) bordered by the terms “no pain” and “the worst pain ever experienced.” Athletes rated their perception of soreness on rising from a seated position by using a sticker to make a mark on the line to indicate their feeling of muscle pain relative to those two extreme points. The mark was then measured as the distance from the left border of “no pain”.

6-2-3. Blood sampling and analysis

Estradiol, progesterone concentration were determined by using a Chemiluminescence Enzyme Immunoassay (CLEIA) and CK/LD levels were analyzed by an independent laboratory (LSI Medience, Ibaraki, Japan).

6-2-4. Statistical analysis

All data are presented as means ± SDs. SPSS version 25.0 (SPSS Inc, Chicago, IL, USA) were used to analyze in group × time using repeated measure ANOVA. Bonferroni’s post hoc test was performed only if F achieved statistical significance ($p<0.05$) and there was no significant variance inhomogeneity. One-way ANOVA was used to analyze the significance in each group (CYC, DYS) over the 3 time points (P1, P3, P5) in the ovarian hormones and 4 time points (P1, P2, P3, P4) in other indexes. Additionally, non-paired t-testing was performed to evaluate the difference between 2 groups at each measurement point, within the level of ovarian hormones in all time points, and within the information of age, height, and menstrual cycle between the groups. P<0.05 was considered as statistically significant.

6-3. Results

6-3-1. Basic characteristics and anthropometrics between groups

Basic characteristics and anthropometric in the CYC and DYS groups are shown in Table 6.2. The length of the menstrual cycle was significantly longer in the DYS group than in the CYC group (p<0.01). Age, height, and other indices shown in the anthropometric data were not significantly different between groups.

Table 6.2 Basic characteristics and anthropometrics

	CYC group (n=18)	DYS group (n=7)
Basic characteristics		
Age (years)	19.6 ± 0.7	19.6 ± 1.5
Height (cm)	161.8 ± 4.8	164.2 ± 2.2
Length of the menstrual cycle (days)	32.4 ± 5.6	69.3 ± 20.6*
Anthropometrics		
Body weight (kg)	56.3 ± 4.0	55.8 ± 4.4
Body fat mass (kg)	13.2 ± 2.4	12.0 ± 2.6
Body water volume (kg)	31.6 ± 1.7	32.1 ± 1.8

* p<0.05 between CYC group and DYS group. The data in anthropometrics are shown as the results assessed in P1.

6-3-2. Time-point comparison in CYC and DYS group, and between groups

6-3-2-1. Ovarian hormone concentration

Ovarian hormones were evaluated at P1, P3, and P5 (Figure 6.2). There was no significant change in the estradiol and progesterone concentrations in either CYC or DYS groups. Estradiol levels were significantly higher in the CYC than in the DYS group at P1, P3, and P5. Progesterone levels were significantly higher in the CYC group than in the DYS group at P1 and P5.

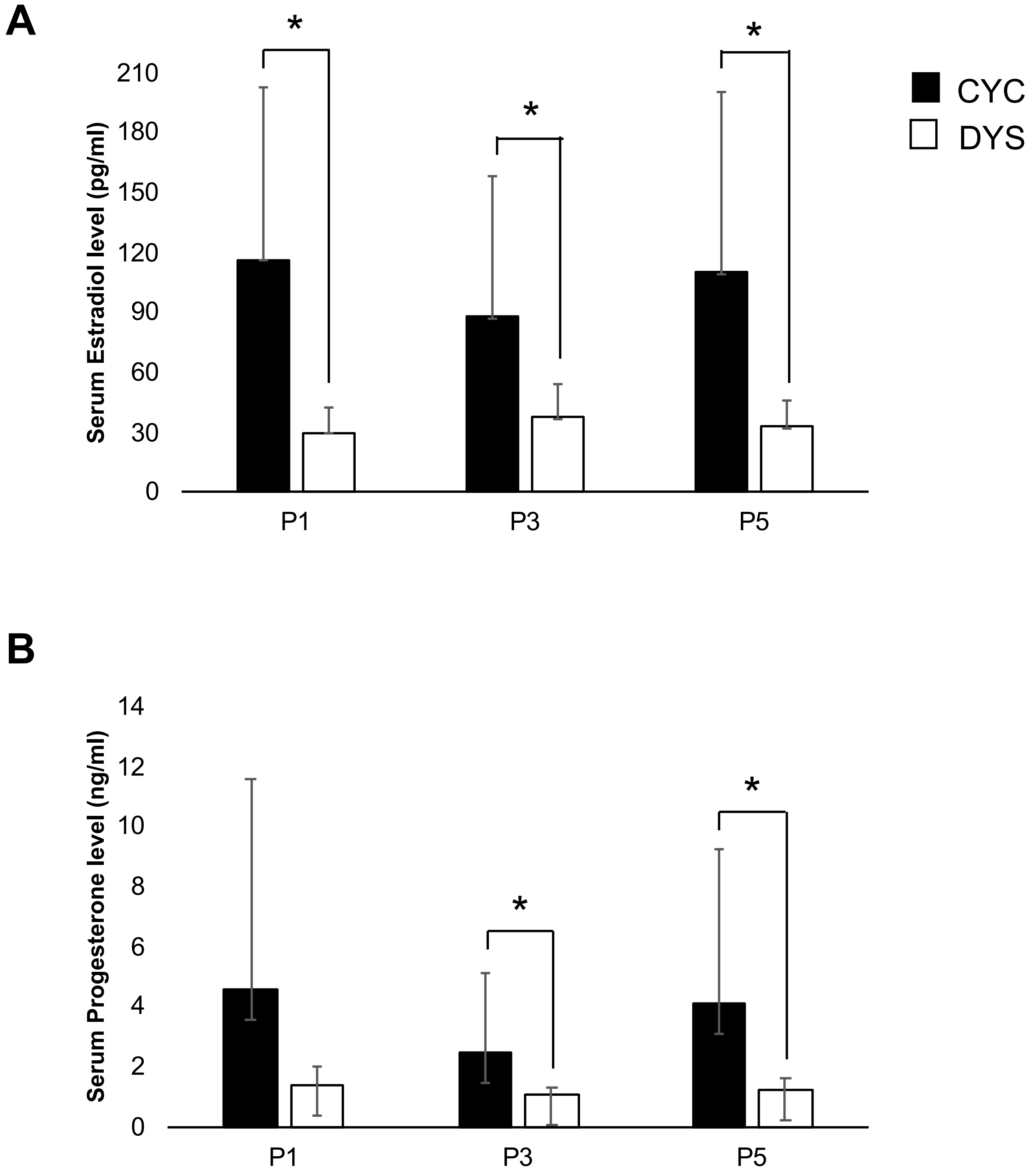


Figure 6.2 Ovarian hormone concentrations

Serum hormone levels in the CYC group are represented by black bars and the DYS group is represented by white bars.

* shows $P<0.05$ between CYC and DYS group in each time-point.

6-3-2-2. Anthropometrics and blood pressure, heart rate

The anthropometric index in the CYC group showed a significant difference only in body weight over the

4 time points (P1-P4). Athletes had significantly high body weights at P2 (57.1±1.0 kg), which was the last day of training camp, compared to P1 (56.3±1.0 kg), P3(56.5±1.0 kg), and P4 (56.4±1.0 kg). Body fat mass, body water volume, systolic blood pressure, diastolic blood pressure, and heart rate did not show significant changes over the timepoints. Within the DYS group, there were no significant differences in body weight and body fat mass. Body water volume was significantly low at P2 (31.0±0.8 kg) compared to P3 (32.6±0.8 kg) and P4 (32.5±0.6 kg).

There were no significant differences in anthropometric data, systolic blood pressure, and heart rate in all timepoints between groups. Diastolic blood pressures in P2, P3, and P4 were significantly lower in the DYS group (70.9±3.2, 70.6±4.2, and 73.1±4.1 mmHg, respectively) than in the CYC group (75.4±6.6, 77.1±7.5, and 79.4±7.4 mmHg, respectively).

6-3-2-3. Serum creatine kinase and lactate dehydrogenase level

Creatine kinase in the CYC group elevated significantly in P2 (344.1±44.8) and P3 (283.8±41.0) compared to P1 (143.8±9.5). Furthermore, P3 (283.8±41.0) and P4 (184.4±27.5) were significantly lower than P2, and P4 was significantly lower than P3. Creatine Kinase in the DYS group was significantly higher in P2 (563.6±92.9) compared to P3 (421.9±72.9) and P4 (244.3±28.4).

Lactate dehydrogenase levels significantly increased in P2 (209.3±9.2) and P3 (202.8±9.1) compared to P1 (179.9±6.6). Furthermore, P3 was significantly higher than P4 (188.2±7.6), and P3 was significantly higher than P4. Lactate dehydrogenase levels significantly increased in P2 (236.1±9.1) compared to P1 (197.6±7.6). Creatine kinase levels in P1(143.8±9.5 and 271.0±32.4 in the CYC and the DYS group, respectively) and P2 (344.1±44.8 and 563.6±92.9 in the CYC and the DYS group, respectively) were significantly higher in the DYS group compared to the CYC group (Figure 6.3). Furthermore, Lactate dehydrogenase level in P4 (188.2±7.6 and 215.4±9.6, respectively) was significantly higher in the DYS group than in the CYC group (Figure 6.4).

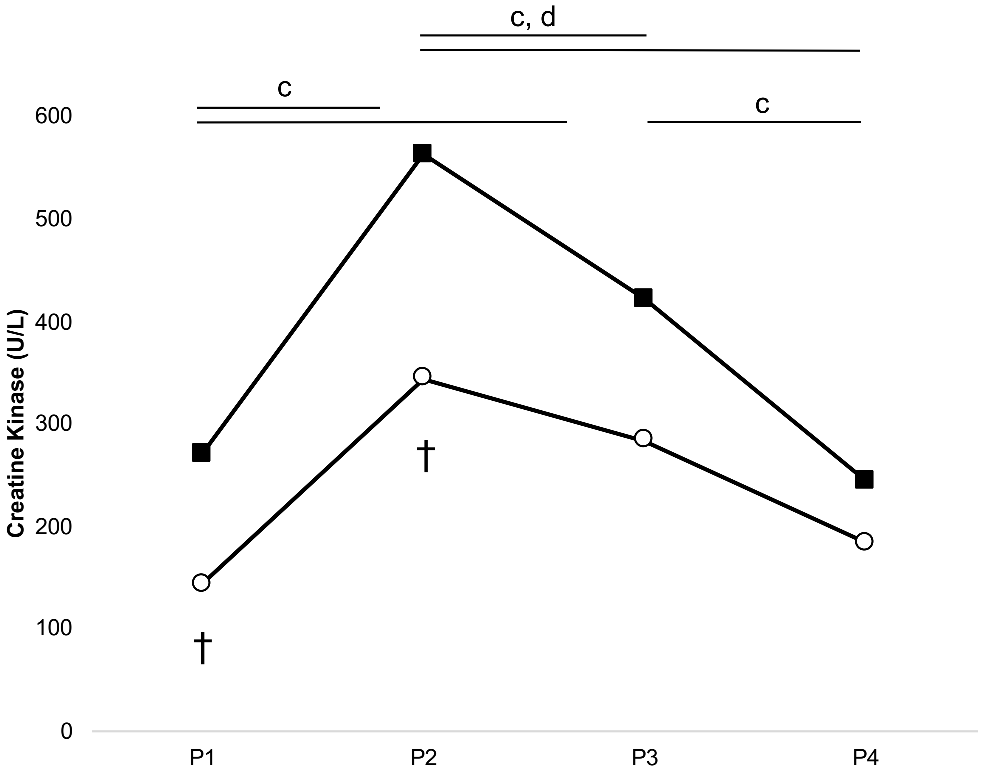


Figure 6.3 Variation of Creatine Kinase level in DYS and CYC group.

The CYC group is shown in white circle markers and the DYS group is shown in black square markers. Standard error bars are omitted for clarity. d and c are shown as $p<0.05$ over time points in DYS and CYC groups. † indicates $p<0.05$ between groups in the same point.

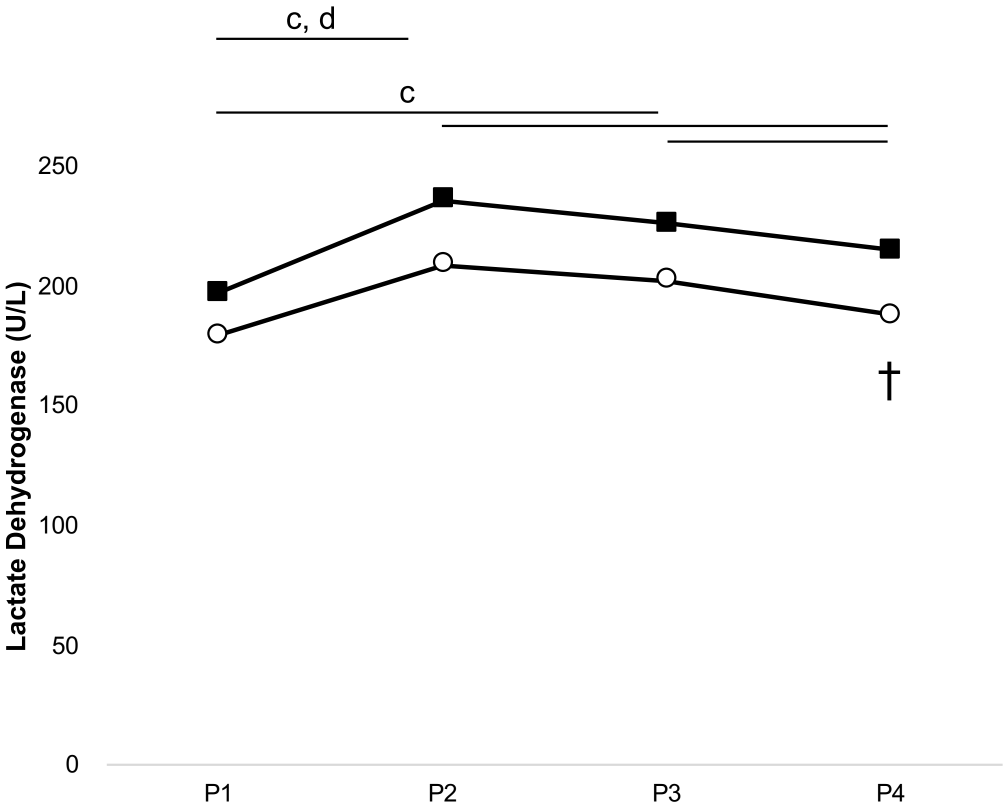


Figure 6.4 Variation of Lactate Dehydrogenase level in DYS and CYC group.
The CYC group is shown in white circle markers and the DYS group is shown in black square markers. Standard error bars are omitted for clarity. d and c are shown as $p<0.05$ over time points in DYS and CYC groups. † indicates $p<0.05$ between groups in the same point.

6-3-2-4. Emotional distress

Emotional distress did not show significant differences over the timepoints in either group. However, TMD scores at P2 and P3 were significantly higher in the DYS group (25.5±17.7 and 19.9±12.4, respectively) than in the CYC group (12.9±10.8 and 9.7±12.5, respectively).

6-3-2-5. Muscle stiffness

The CYC group showed a significant difference only in biceps femoris muscle stiffness over the timepoints. Biceps femoris stiffness at P2 (45.1±4.1) and P3 (45.9±3.4) were significantly higher than P1 (31.9±3.0) and P4 (29.8±3.0). In the DYS group, muscle stiffness in the biceps femoris was elevated significantly from P1(41.0±1.8) to P2(55.5±4.2) and P3(58.6±3.4). Furthermore, P3 was significantly higher than P4(37.9±1.7). Muscle stiffness in the gastrocnemius also showed a significant difference. P3(53.8±2.5) was significantly higher than P1(43.6±1.3) and P4(41.9±1.0). Biceps femoris muscle stiffness in P3 and P4 were significantly higher in the DYS group (58.6±9.7 and 37.9±4.9, respectively) than in the CYC group (45.9±13.9 and 29.8±12.5, respectively). Furthermore, stiffness of gastrocnemius in P2 and P3 were significantly higher in the DYS group (51.4±9.9 and 53.8±7.0, respectively) than in the CYC group (38.6±14.2 and 45.2±7.7).

6-3-2-6. Muscle soreness

Muscle soreness in the CYC group increased significantly from P1(33.4±4.3) to P2(69.9±3.0), P3(69.1±3.0), and P4(57.2±4.2). In DYS, the soreness significantly elevated in P2(77.1±1.7) and P3(69.8±3.5) compared to P1(40.4±5.1). Furthermore, P2 was significantly higher than P4(60.4±2.9).

6-4. Discussion

Two participants in the DYS group had a menstruation delay for more than 3 months, indicative of amenorrhea. The other 6 participants did not show a menstruation delay for more than 3 months, however, their menstrual lengths (days) were significantly longer than the CYC group and showed a prolonged low level of ovarian hormones over the measurements. Furthermore, the DYS group did not show significant differences in body weight and body fat mass compared to the CYC group. Athletes in low body weight, high intensity training, aesthetic sport, weight classifications and long-distance runners are reported to have a risk of exercise-related amenorrhea (Redman et al., 2005). This has been previously reported in athletes with amenorrhea or oligomenorrhea as being characterized by low body weight, and low body fat volume (Nattiv et al., 2007; Mountjoy et al., 2014; Wojtys et al., 2015; Joy et al., 2016) which was not seen in this study. Previously, the IOC introduced a more comprehensive, broader term for the overall symptoms comprising the female athlete triad called “Relative Energy Deficiency in Sports (RED-S) and suggest health consequences, including menstrual dysfunction, lead to negative effects on athletic performance (Mountjoy et al., 2007; De

Souza et al., 2014). According to this menstrual status in female athletes, the American College of Sports Medicine suggested that emphasis should be placed on optimizing energy availability for prevention by increasing body weight and body fat, reducing training volume, and HRT (Stand position 2007). However, DYS athletes reported to have a menstrual cycle 1 year prior to the study (albeit less than 5 cycles per year) so heavy training seemed to result in frequent cycle delays which is in line with previous reports on amenorrhea (Jordan et al., 1994; Baxter-Jones et al., 1994; Dušek 2001 and 2004; Redman et al., 2005).

Emotional distresswas significantly different between DYS and CYC groups at P2 and P3 (DYS group was significantly higher) while no significant differences were seen over the 5 timepoints in each group. It has been reported in amenorrheic athletes that their TMD scores were significantly higher than eumenorrheic athletes, scoring higher in “tension” “depression” “anger” “fatigue” “confusion”, and a difference was seen in eumenorrheic athletes reporting that TMD scores were significantly lower 14 days after menstruation compared to the pre-menstruation period (Cockerill et al., 1992). Furthermore, women with functional hypothalamic amenorrhea have been reported to have higher scores in interpersonal dependence compared to eumenorrheic women and have histories of psychiatric disorders, primarily mood disorders, more often than eumenorrheic women (Giles et al.,1993). Additionally, depression scores improved after estrogen replacement therapy in postmenopausal women (Soares et al., 2001; Rasgon et al., 2002; Cohen et al., 2003). Our participants in the DYS group were not all diagnosed as (exercise-induced) amenorrheic; however, the TMD scores increased significantly compared to the CYC group especially during the day when participants showed a greater muscle soreness in P2 (the last day of the training camp). It could be possible that the greater scores of muscle soreness in the DYS group was related to the TMD scores and lasted to the next day. Moderate intensity training has been reported to be associated with a positive mood (Berger et al., 2000) while intense training increases negative moods, including anxiety, fatigue, and lack of vigor (Berger et al., 1999).

In previous reports focusing on sex differences, muscle soreness peaked in 24 hours after downhill exercise in both men and women in the follicular phase, though the soreness in women did not recover to the pre-exercise level 72 hours after exercise while men did (Oosthuyse et al., 2017). However, other reports showed no significant sex differences in muscle soreness 4, 7, and 10 days after exercise (Sewright et al., 2008). Over the menstrual cycle, it has been reported that the recovery of muscle soreness was faster in mid-luteal phase than in early follicular phase and late follicular phase (Oosthuyse et al., 2017). In fact, estrogen and progesterone receptors are expressed on central and peripheral nerves and have been reported to induce both pronociceptive and antinociceptive affects where the overall effect may be dependent on the receptor type and relative presence of estrogen and progesterone (Iacovides et al., 2015). It has been reported that estrogen has antinociceptive effects (Aloisi et al., 2010) and may reduce sensory neurotransmission via down-regulation or inhibition of transient receptor potential vanilloid subfamily 1 receptors (Xu et al., 2008). Few studies have reported that high or fluctuating levels of estrogen can enhance afferent sensory input through glutaminergic mechanisms (McRoberts et al., 2007) and pronociceptive actions via inflammatory and stress responses. However still, the influence of the menstrual cycle on pain perception is complicated and not clearly described (Iacovides et al., 2015).

CK is well known for its role in muscle disruption, where it peaks around 24-72 hours and later than CK in LD (Brancaccio et al., 2007; Deminice et al., 2011). CK level has been reported to increase significantly 24 hours after downhill running in early-follicular phase (EF), late-follicular phase (LF), and mid-luteal phase (ML) though no significant differences between the phases was observed. It is possible to consider that estrogen does play a protective role in the modulation of CK levels after heavy exercise over the menstrual cycle, though the coincident increase in progesterone with estrogen seen in week 3 may modulate the protective effect of estrogen, which was similarly considered in a previous report (Thompson et al., 2006). There is one report showing a higher basal CK level in amenorrheic athletes than eumenorrheic athletes (Thompson et al., 2006); an effect similarly seen in Chapter 6. Estrogen has been previously reported to play a protective role in muscle as a muscle membrane stabilizer, mediator of leukocyte infiltration and marker of tissue disruption following injury (Tiidus et al., 1999, Stupka et al., 2000; Tiidus 2001). Furthermore, estrogen's membrane stabilizing effect to reduce the intracellular calcium influx promotes calpain protease activity leading to increased chemoattractants and neutrophil infiltration after exercise (Tiidus et al., 1999; Tiidus et al., 2001; Enns et al., 2010). It is also possible that the protective effects of estradiol on muscle damage are mediated predominantly by estradiol-α receptors, rather than the β receptors measured in the present study (Thor et al., 2010) which both receptors express on mammalian skeletal muscle (Kalbe et al., 2007). Although α and β receptors differ among tissue types and species with the β often attributed for immune modulation, recent animal model work reveals that the α receptor is important following exercise-induced muscle damage (Thomas et al., 2010). Fortunately, in this study, the measurement protocol was able to assess up to 48 hours after the training camp in both groups, with the DYS group showing higher levels in serum CK levels in P1 and P2 compared to CYC. It has been previously reported that estrogen supplementation in rats showed activation and proliferation in satellite cells (Enns et al., 2008) through the binding of estrogen and estrogen receptors, which the expression of increases after exercise (Lemoine et al., 2002). The binding of estrogen and estrogen receptors is previously reported to promote the protein synthesis and enhancement of skeletal muscle through the PI3 kinase Akt signaling pathway (Patten et al., 2004; Sitnick et al., 2006). However, although serum CK and LD may not reflect exercise-induced muscle damage directly, it is still commonly used to serve as an indirect index of exercise-induced muscle damage in previous reports (Yanagisawa et al., 2011a; Guilhem et al., 2013; Koch et al., 2014; Rowlands et al., 2015), suggesting that the observed basal CK levels were higher in the DYS than in the CYC group on the last day of the training camp but recovered similarly to the CYC group.

The muscle stiffness of the biceps femoris was elevated in P2 and P3 compared to P1 and significantly decreased in P4 compared to P2 and P3 in both DYS and CYC groups. Gastrocnemius muscle stiffness did not show a significant change over the measurement in the CYC group while P3 in the DYS group was significantly higher than P1, P4, and P5. Furthermore, biceps femoris and gastrocnemius stiffness were significantly higher in the DYS group in P3, P4 and P2, P3 respectively compared to the CYC group. Estrogen receptors have been identified within skeletal muscle (Lemoine et al., 2003) and are believed to modulate muscle strength (Sarwar et al., 1996), metabolism (Hackney et al., 1999), and stiffness observing a decrease in lower extremity stiffness at ovulation which is a phase accompanies by appreciable increases in estrogen (Eiling et al., 2007). Estrogen may play a role, because estrogen receptors are present in fibroblasts of tendons and ligaments, which may alter collagen synthesis and affect tissue behavior (Kjaer et al., 2008). However, the equipment which was used in this study was percutaneous, suggesting that the elevation of stiffness should also take swelling into account (Chleboun et al., 1998). In general, neutrophils infiltrate damaged muscle within several hours after eccentric exercise and are replaced by blood monocyte-derive macrophages within approximately 24h post-exercise (Peake et al. 2005). The infiltration of inflammatory cells from the blood vessel is accompanied by fluid effusion (Peake et al. 2005). In addition, various cytokines that act as inflammatory mediators that increase vascular permeability are produced in the damaged muscle within several hours after repeated eccentric contraction (Paulsen et al. 2005). However, it has been reported that estrogen plays a protective role against the efflux of CK into the extracellular space after the stimulation of exercise, which activates calpain and promotes the decomposition of protein, leukocyte infiltration and markers of tissue disruption (Tiidus et al., 1999; Tiidus 2000; Enns et al., 2010). Therefore, the significant differences seen between the groups from the last day of the training camp may be due to the lack of estradiol in the DYS group.

To summarize, the DYS group, which was characterized by chronically low estrogen and progesterone levels, led to increased muscle damage and negative emotional distress after an intensive training camp. Further mechanistic studies are needed to explain this process in detail; however, the presence of sufficient ovarian hormones and their protective role in preventing exercise-induced damage has been examined in this study.

7. General discussion

7-1. Introduction and key findings

The skyrocketing participation of female athletes in competitive sports has underlined the importance of recognizing sex differences. However, the effect of sex hormones on competitive performance and conditioning have not been extensively studied. The aim of the research studies presented in this thesis was to investigate the effect to the competitive performance and conditioning under different ovarian hormone levels in women.

The main findings are summarized below:

- Menstrual phase (which is characterized as low estradiol and low progesterone level with bleeding) in eumenorrheic women showed a high-water retention in the lower legs compared to other phases by using MRI. Furthermore, static balance ability and agility were worse in the menstrual phase compared to other phases (Chapter 3).
- The volume of water retention and agility showed a negative correlation in the menstrual phase in eumenorrheic women (Chapter 3).
- Collegiate female athletes experience the FAT risk factors in a manner correlated to the intensity of their sports type and competitive level (Chapter 4).
- Correlation was seen between lower competitive levels and delayed menarche / amenorrhea. However, musculoskeletal injuries and nutrition choice had no ties to the competitive level (Chapter 4).
- Athletes with menstrual disturbances who were characterized with chronically low ovarian hormone level showed a higher and prolonged muscle damage response (CK levels and soreness) after acute heavy exercise than in eumenorrheic athletes. Furthermore, biceps femoris tendon stiffness significantly increased 24 hours after exercise in the group with menstrual disturbances while eumenorrheic athletes did not show significant changes (Chapter 5).
- Athletes with menstrual disturbances, characterized by chronically low ovarian hormone levels,

showed greater muscle damage response and negative emotional scores on the last day of a 4-day training camp, as well as 1 and 2 days after the camp, compared to eumenorrheic athletes (Chapter 6).

The following discussion will analyze these findings in respect to other research.

7-2. Cyclic fluctuation of ovarian hormones in the regular menstrual cycle and its effect on athletic performance

The menstrual cycle can be divided into several phases according to the ovarian hormone concentrations. Following Chapter 3, the menstrual phase which is characterized by low levels of both estrogen and progesterone decreases static balance ability and agility while increasing water retention in the lower calf.

Menstrual cycling is regulated by estrogen and progesterone which fluctuates rapidly and cyclically, and it has been reported previously that hormone levels can affect athletic performance (Lebrun et al., 1993). Hayashi et al reported that static balance ability decreased significantly in the menstrual, early- luteal, and late-luteal phases compared to the follicular phase and the locus length per second decreased significantly in the late-luteal phase (Hayashi et al., 2004), suggesting the secretion of estradiol and progesterone as a possible cause. These hormones are also reported to have a central nervous system effect, potentially affecting posture control indirectly (Posthuma et al., Lebrun et al., 1994; Woolley 1999). Female soccer athletes showed a longer record in the Yo-Yo intermittent endurance test in follicular phase (which is characterized as low ovarian hormone level with no bleeding) compared to the luteal phase (which is characterized as high ovarian hormone levels in both estrogen and progesterone). The body temperature elevation which is usually seen in the luteal phase has been suggested to limit prolonged exercise capabilities and increase cardiovascular strain (Janse de Jonge 2003). However, menstrually related symptoms can also be a factor in reducing athletic performance and conditioning in women. About 80% of Japanese top-level female athletes in Japan experience menstrual discomfort (Nakamura 2012). Symptoms which are mostly associated with localized pain have been reported to reduce athletic performance in eumenorrheic athletes. Approximately 44% of Japanese collegiate athletes were found to suffer from premenstrual syndrome (including menstrually related pain) in games and in practice (Takeda et al., 2015). Muscle strength decreased both in luteal phase and during menstruation in athletes with dysmenorrhea compared to athletes with no symptoms (Martínez-Cantó et al., 2018) and, furthermore, jump ability decreased significantly in the menstrual phase compared to the follicular phase in athletes complaining of menstrually related pain (Giacomoni et al., 2000). However 10% (of the aforementioned 80%) are also afflicted by edema (Nakamura 2012) in both premenstrual and menstrual phases where the effect on athletic performance has not been clearly explained. Our findings also support the suggestion that this edema could be the result of a delayed response to previously higher hormone levels (White et al., 2011). The reason for this still remains unclear; however, the increase in serum hormone levels and the appearance of the edema with a 3-4 days delay is notable.

Although participants of the present study did not complain of any typical PMS symptoms, the results show that fluid retention increased significantly during the menstrual phase. T2 signal changes during the menstrual cycle showed fluid retention when the levels of estrogen, progesterone, and aldosterone were the lowest, which contradicts the main theory of premenstrual edema. Furthermore, water retention elevation was negatively correlated to agility in our study. Further mechanism on this result still needs further investigation. In female athletes, it may be hard to discriminate whether the variation of water retention occurs due to either sex hormone fluctuations or as a response to exercises and, at this stage, healthy individuals were included in our study. Recruitment of female athletes along with women with normal physical activity levels will be favorable for this project, which at the moment is a first step in accumulating base-line data for further investigation of the phenomenon in athletes.

7-3. Chronic low ovarian hormones in athletes with menstrual disturbances and its influence on athletic performance

Nose et al. (2014) have reported that over 40% of Japanese top-level female athletes suffer with menstrual irregularities while 59.9% are with normal menstrual cycle. Especially in athletes with prolonged low ovarian hormone such as amenorrhea has been reported to have a higher risk of injuries (especially bone stress fracture) (Nose et al., 2014), higher negative score in psychological evaluation (Cockerill et al., 1992), low level of allopregnanolone and high level of cortisol and ACTH in the basal level compared to eumenorrheic women (Meczekalski et al., 2000), low level of salivary SIgA secretion and higher appearances of upper respiratory tract infection (URTI) symptoms than eumenorreic athletes (Shimizu et al., 2012). Previously, IOC introduced a more comprehensive, broader term for the overall symptoms consisting female athlete triad called "Relative Energy Deficiency in Sports (RED-S) and suggest health consequences including menstrual dysfunction, leading to negative effect to the athletic performance (Mountjoy et al., 2014; De Souza et al., 2014). According to this menstrual status in female athletes, American college of sports medicine suggested that emphasis should be placed on optimizing energy availability for prevention by increasing body weight and body fat, reducing training volume, and HRT (Nattiv et al., Stand position 2007). However, it is a hard and unacceptable issue for athletes to reduce their training volume and gain weight for even a short term over a year, especially in aesthetic athletes. Furthermore, the medical treatment such as hormone replacement therapy and oral contraception will be a major burden for athletes in financial and time restriction and is not easy to continue for many years. Therefore, it is necessary not only in athletes but other supporters (i.e. trainers, coaches) to understand the influence of the menstrual status on their physical condition and competitive performance, and share the information of it to suggest a way that can be done in the sports field which was shown in Chapter 5.

Estrogen and progesterone are previously known as an ovarian hormone to regulate the menstrual cycle. Especially, estrogen has been reported to have positive 1) psychological (Cockerill et al., 1992), 2) physiological (Meczekalski et al., 2000), and 3) immunological (Shimizu et al., 2012) effect to female athletes, and effect improving athletic performance such as static balance ability (Hayashi et al., 2004), jumping ability (Giacomoni et al., 2000; Bryant et al., 2008), and agility (Rashmi et al., 2017) when estrogen is in a high level. Estradiol has been previously reported to play an important role in membrane stabilization (Claassen et al., 2005) and antioxidant (Enns et al., 2010) in vivo and in vitro. Additionally, estrogen is reported to suppress the elevation of Creatine Kinase (CK) level and histological muscle damage after exercise in animals, which has focused in sex difference (Amelink et al., 1990; Komulainen et al., 1999; Tiidus et al., 2001). Studies in men have investigated that basal CK level is significantly higher in male (who are basically in low estrogen level) than female (Mougois et al., 2007). One previous study has reported greater CK level in men than women after eccentric resistance exercise following 4, 7 and 10 days after with the earlier recovery time points omitted (Sewright et al., 2008), which is impractical for athletes who do not constantly have a long-term activity rest. Resting serum CK level has previously been reported to be higher in amenorrheaic athletes who persistently have low estrogen level, than in eumenorrheic athletes (Thompson et al., 2006). However, this study has not evaluated the CK response after exercise. Study in postmenopausal women have reported to have higher CK level after eccentric exercise in women who had not taking hormone replacement therapy than in women who was (Dieli-Conwright et al., 2009). Similarly, CK level in eumenorrheic athletes' late follicular phase (which only estrogen rapidly increases) significantly decreased following 24 hours after downhill exercise compared to early follicular phase and middle-luteal phase over the menstrual cycle (Oosthuyse et al., 2017). Studies focusing on the response of response after exercise have been reported previously, though do not consider the menstrual phase or menstrual status in their studies (i.e., eumenorrhea, amenorrhea, oral contraceptive use, postmenopausal, etc.) (Sorichter et al., 2001; Sewright et al., 2008), or just measure in only one phase (Stupka et al., 2000; Minahan et al., 2015). The protective effect of estrogen against muscle following heavy exercise has been previously reported in many studies as mentioned above, however the muscle damage response following heavy exercise in ovarian suppressed athletes (who are characterized as persistently low ovarian hormone level) and the difference to eumenorrheic athletes has not been known clearly. Therefore, Chapter 6 and 7 firstly show the acute and chronic exercise effect to the physical condition, especially in muscle damage which suggested the greater response in menstrual dysfunction athletes characterized with chronically low ovarian hormones concentration.

Chapter 8. General conclusion

Through these four studies shown in this thesis, the effect of the cyclic variation of ovarian hormones in eumenorrheic women on water retention has been correlated to athletic performance. Furthermore, athletes with menstrual disturbances, characterized as chronically low ovarian hormones, showed greater muscle damage after acute and prolonged exercise in athletes.

As a general conclusion, chronically-low ovarian hormone status brings both negative competitive performance and condition to female athletes, evidenced by low competing levels and a delay in muscle damage markers after exercise. Eumenorrheic athletes were shown to be affected by their cyclic hormones, but with much less effect than the ovarian-suppressed athletes. Therefore, in eumenorrheic athletes, it can be suggested that maintaining their menstrual cycles and considering the effect of each of the phases will be more useful to plan the peaks in their athletic condition.

8-1. Directions for future research

From Chapter 4 to Chapter 7, the effect of the variation of ovarian hormones on athletic performance and conditioning have been performed in non-active women and female athletes. However, further details on estradiol’s protective role and its effect on physiological conditioning still needs to be investigated to explain the mechanisms shown in these studies.

Furthermore, chronically low sex hormone concentrations and increased risk of other health issues among athletes has been reported to occur in males. The correlation of sex hormones to competitive performance should be investigated for the prevention of severe health issues not only in female but also male athletes before consequences are too difficult to reverse.

8-2. Practical applications

Female athletic performance was shown to vary through the regular cyclic menstrual cycle in Chapter 3. It will be useful for athletes and coaches, trainers, and medical doctors to control and compensate for menstrual cycling to avoid phases which were reported to show a decrease in athletic performance and further avoid the effect of menstrually related symptoms. However, most collegiate female athletes experience short-term menstrual irregularities which was shown in Chapter 4. The American College of Sports Medicine suggests improving low energy availability status, however changing ingrained habits and altering the regimen of diets and exercise could be quite difficult for athletes, especially in endurance sports, aesthetic sports, and weight-classified sports athletes. Additionally, Chapter 5 and Chapter 6 showed that chronic menstrual irregularities were associated with higher and prolonged muscle damage response (CK levels and soreness) after acute and prolonged exercise. Therefore, coaching staff, athletic trainers, and doctors should consider an athlete's individual menstrual status with respect to exercise therapy and training menus to minimize musculoskeletal damage and injury risk.

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