Effects of intensive training on menstrual function and certain serum hormones and peptides related to the female reproductive system

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Abstract

The aim of this study was to assess the effects of intensive training on menstrual function and related serum hormones and peptides.

Forty female participants who attended a training course for an officer at the Korea Third Military Academy, and had regular menstrual periods were enrolled. Menstrual questionnaires and fasting blood samples were collected before entry and at 4-week intervals for 8 weeks. The levels of corticotropin-releasing hormone (CRH), cortisol, prolactin, endorphin-β, neuropeptide Y (NPY), leptin, orexin-A, ghrelin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E$_2$), thyrotropin (TSH), and thyroxine (T$_4$) were determined.

Body mass index and waist circumference decreased during the training course. Intensive training of military cadets resulted in changes of menstruation and related biomarkers. The levels of CRH, endorphin-β, NPY, orexin-A, ghrelin, E$_2$, and T$_4$ decreased substantially, and cortisol, prolactin, and TSH increased. Seventy percent of participants with regular menstrual periods before developed irregular during the training course. Participants were then categorized into 2 groups: those with regular menstruation (n=12) and those with irregular menstruation (n=28). The levels of hormones and peptides were not different between the 2 groups.

In conclusion, cortisol, prolactin, and TSH level increased but levels of CRH, endorphin-β, NPY, orexin-A, ghrelin, E$_2$, and T$_4$ decreased throughout the training. Moreover, the levels were not different between participants with normal menstruation and those with irregular menstruation. Further research should extend these findings by investigating the exact mechanism by which high exercise levels, including intensive training, interfere with regular menstruation.

Abbreviations: AVP = arginine-vasopressin, BMI = body mass index, CRH = corticotropin-releasing hormone, E$_2$ = estradiol, FSH = follicle-stimulating hormone, GnRH = gonadotropin-releasing hormone, HPA = hypothalamic-pituitary-adrenal, LH = luteinizing hormone, NPY = neuropeptide Y, OC = oral contraceptive, T$_4$ = thyroxine, TSH = thyrotropin, WC = waist circumference.

Keywords: exercise, hormone, menstruation, peptide

1. Introduction

Although regular exercise has been associated with many positive effects on body function, such as improved insulin sensitivity,[1] decreased visceral adipose tissue mass, and improvements in

cardiovascular function,[2] intense exercise has been reported to cause luteal phase defects (oligomenorrhea and other menstrual dysfunctions) and amenorrhea.[3] Many studies have confirmed that intense exercise causes oligomenorrhea and amenorrhea in athletes,[4] and moderate exercise was associated with a slightly increased probability of longer cycles,[5] as undesirable side effects of exercise that offset its positive effects. Various hormones and peptides are known to have important roles in the modulation of menstrual regularities. However, the exact physiological changes of these hormones and peptides remain unclear, and different researchers have come to variable conclusions about the association between these substances and menstrual irregularities, because contributing physiological and psychological factors such as intensity of exercise, calories, stress, and sleep duration produce considerable individual variations.[6,7]

There are several studies about exercise-induced menstrual pattern changes in restricted environments or in age-specific populations. For example, some studies reported that 68% to 98% of cadets who had regular menstrual periods before training became irregular.[8,9] Understanding the menstrual function of women undergoing physical training programs held in the army has practical importance because they are a unique population in whom some bias factors related to menstrual function, such as diet and sleep duration, could be controlled.

The aim of this study was to investigate the effects of intensive training on various hormones and peptides related to the reproductive system and further investigate the association of these substances with menstrual regularity in Korean healthy young women.

2. Methods

Female cadets who participated in a 16-week training course held at the Korea Military Academy were invited to participate in this study. Among 183 entering female cadets, 70 volunteered to participate. All participants gave written informed consent for participation in the study, which was approved by the clinical research ethics committee. Questionnaires about medical history and physical activity were collected before entry into the course.

Menstrual questionnaires and anthropometric parameters were collected before entry into the course and 2 more times during the next 8 weeks at a 4-week interval.

Menstrual regularity was assessed on the basis of the questionnaire answer to, "How many days had passed from the 1st day of 1 bleeding to the 1st day of the next one?" The periods were defined as regular if the overall range was within 21 to 35 days, or if the difference between the shortest and the longest cycle was <15 days.[10,11]

Twenty volunteers were excluded because of an irregular menstruation pattern and use of oral contraceptives (OCs) or other hormone therapy before entry. Seven who were taking OCs for menstrual control during training and 3 who were hospitalized or dropped out of the training course were also excluded. Ultimately, 40 participants were enrolled in the study.

Body mass index (BMI; kg/m^2) was calculated using measured weights and heights. Waist circumference (WC) was measured during minimal respiration from the narrowest point between the borders of the rib cage and the iliac crest.

Fasting blood samples were collected before entry into the course and 2 more times during the next 8 weeks at a 4-week interval. Blood sample was drawn at 6:00 to 7:00 AM after an overnight fast prior to beginning training for the day from the antecubital vein into a centrifuge tube that contains no anticoagulate. Samples were allowed to clot for 30 minutes at room temperature and then centrifuged at 2000 rpm for 15 minutes at 4 °C. Then, serum samples were aliquoted into cryovials and stored at −70 °C until analysis. Hormones and peptides, which are known to be changed and related to reproductive systems in female athletes, were selected to be measured in this study.[12] Levels of serum corticotropin-releasing hormone (CRH), cortisol, prolactin, endorphin-β, neuropeptide Y (NPY), leptin, orexin-A, and ghrelin, which are substances known to be related to the reproductive system, and follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E_2), thyrotropin (TSH), and thyroxine (T_4) were determined. Cortisol, prolactin, FSH, LH, and E_2 were measured using a chemiluminescent immunoassay method. CRH, endorphin-β, NPY, and orexin-A were measured using an enzyme-linked immunosorbent assay method. Leptin and ghrelin levels were measured using a radioimmunoassay method. TSH and T_4 were measured using an electrochemiluminescence immunoassay method. The mean intra-assay coefficients of variation ranged from 1.5% (prolactin) to 15% (E_2), and the interassay ranged from 3.7% (T_4) to 15.0% (orexin-A).

The participants that attended this Military Academy course were provided with the basic and specialized physical training. All participants underwent basic physical training 6:30 to 7:30 AM. Basic physical training involved a free gymnastics and a running for a distance of 1 to 2 km (Monday to Saturday). The specialized physical training for participants involved many different types of exercise such as ranger training, March, field training exercise, close-order drill, and bayonet exercise (Monday to Friday).

Values are expressed as means (SD). The data were analyzed at 3 time points: baseline, 4 weeks, and 8 weeks. One-way repeated measures analysis of variance (ANOVA) was used to determine whether hormones and peptides differed significantly across time

Table 1

Anthropometric measurements of participants during an 8-week training course.

	Baseline	4 weeks	8 weeks	P*
Weight, kg	63.6 (7.8)	60.0 (6.8)†	59.3 (6.4)†,‡	<.01
BMI, kg/m^2	24.1 (2.7)	22.7 (2.30)†	22.4 (2.2)†,‡	<.01
Waist circumference, cm	71.5 (7.0)	67.0 (5.8)†	67.1 (4.6)†	<.01

Values are expressed as means with SD. ANOVA = analysis of variance, BMI = body mass index, SD = standard deviation.
* P-value was calculated by repeated measures ANOVA.
† P < .05 compared with levels at baseline (post-hoc Bonferroni).
‡ P < .05 compared with levels at 4 weeks (post-hoc Bonferroni).

periods. Where a significant difference occurred, Bonferroni post-hoc analyses were performed. Two-way repeated measures ANOVA were also used to analyze hormones and peptides to assess both the effect of time and group. An alpha level of P < .05 was considered significant. All statistics were performed with SPSS (version 11.0, Chicago, IL).

3. Results

The participants' ages ranged from 22 to 28 years. Table 1 shows the changes of anthropometric parameters during the study periods. Weight and BMI decreased progressively from baseline throughout the training course on repeated measures ANOVA. WC decreased significantly in the 1st 4 weeks of course and then leveled off with no further decrease at 8 weeks.

Table 2 shows the changes of hormones and peptides during the training course. The results of repeated measures of ANOVA showed that intensive training induced a decrease in CRH, endorphin-β, NPY, orexin-A, ghrelin, E_2, and T_4 levels but an increase in cortisol, prolactin, and TSH levels throughout training period. However, there was no change in leptin, FSH, and LH levels. On post-hoc analysis, CRH and ghrelin levels did not change in the 1st 4 weeks but decreased after 8 weeks of the training of course. Cortisol and prolactin levels increased significantly in the 1st 4 weeks and then leveled off with no further increase at 8 weeks. Endorphin-β and NPY levels decreased and remained significantly decreased at the 8-week time point. Orexin-A levels decreased significantly in the 1st 4 weeks and then leveled off with no further

Table 2

Levels of hormones and peptides of participants during an 8-week training course.

	Baseline	4 weeks	8 weeks	P*
CRH, pg/dL	84.4 (65.1)	57.7 (28.3)	22.0 (21.7)†,‡	<.01
Cortisol, μg/dL	16.1 (3.9)	18.1 (2.2)†	18.7 (2.2)†	<.01
Prolactin, ng/mL	11.8 (5.5)	15.5 (7.5)†	17.0 (6.8)†	<.01
Endorphin-β, pg/dL	59.4 (40.8)	42.8 (15.5)†	25.1 (20.9)†,‡	<.01
NPY, ng/mL	2.7 (1.4)	1.6 (1.0)†	0.7 (0.5)†,‡	<.01
Leptin, ng/mL	5.0 (2.7)	5.2 (3.2)	5.0 (3.1)	.64
Orexin-A, pg/mL	225.0 (175.9)	69.3 (61.5)†	56.5 (61.0)†	<.01
Ghrelin, ng/mL	1.1 (0.1)	1.0 (0.1)	1.0 (0.1)†,‡	<.01
FSH, mIU/L	7.0 (3.1)	8.2 (2.8)	8.1 (3.2)	.06
LH, mIU/L	5.4 (8.6)	5.2 (4.3)	4.9 (4.7)	.86
E_2, pg/dL	106.0 (120.7)	44.6 (24.4)†	55.1 (43.1)	<.01
TSH, μIU/mL	2.3 (1.2)	2.2 (1.2)	3.1 (1.3)†,‡	<.01
T_4, μg/dL	7.5 (1.3)	7.1 (1.0)†	7.0 (1.0)†	<.01

Values are expressed as means with SD. ANOVA = analysis of variance, BMI = body mass index, CRH = corticotropin-releasing hormone, E_2 = estradiol, FSH = follicle-stimulating hormone, LH = luteinizing hormone, NPY = neuropeptide Y, OC = oral contraceptive, SD = standard deviation, T_4 = thyroxine, TSH = thyrotropin, WC = waist circumference.
* P-value was calculated by repeated measures ANOVA.
† P < .05 compared with levels at baseline (post-hoc Bonferroni).
‡ P < .05 compared with levels at 4 weeks (post-hoc Bonferroni).

decrease at 8 weeks. TSH levels did not change in the 1st 4 weeks but increased after 8 weeks of the training of course. T_4 levels decreased significantly in the 1st 4 weeks and then leveled off with no further decrease at 8 weeks.

Seventy percent of participants (n=28) who had regular menstruation before attending the training course developed irregular during the training course. Participants were then categorized into 2 groups according to their menstrual status during the training course as follows: regularly menstruating group (n=12) and irregularly menstruating group when the menstrual cycle showed disruption whenever within the 8 weeks course (n=28). Figure 1 shows the changes in the levels of
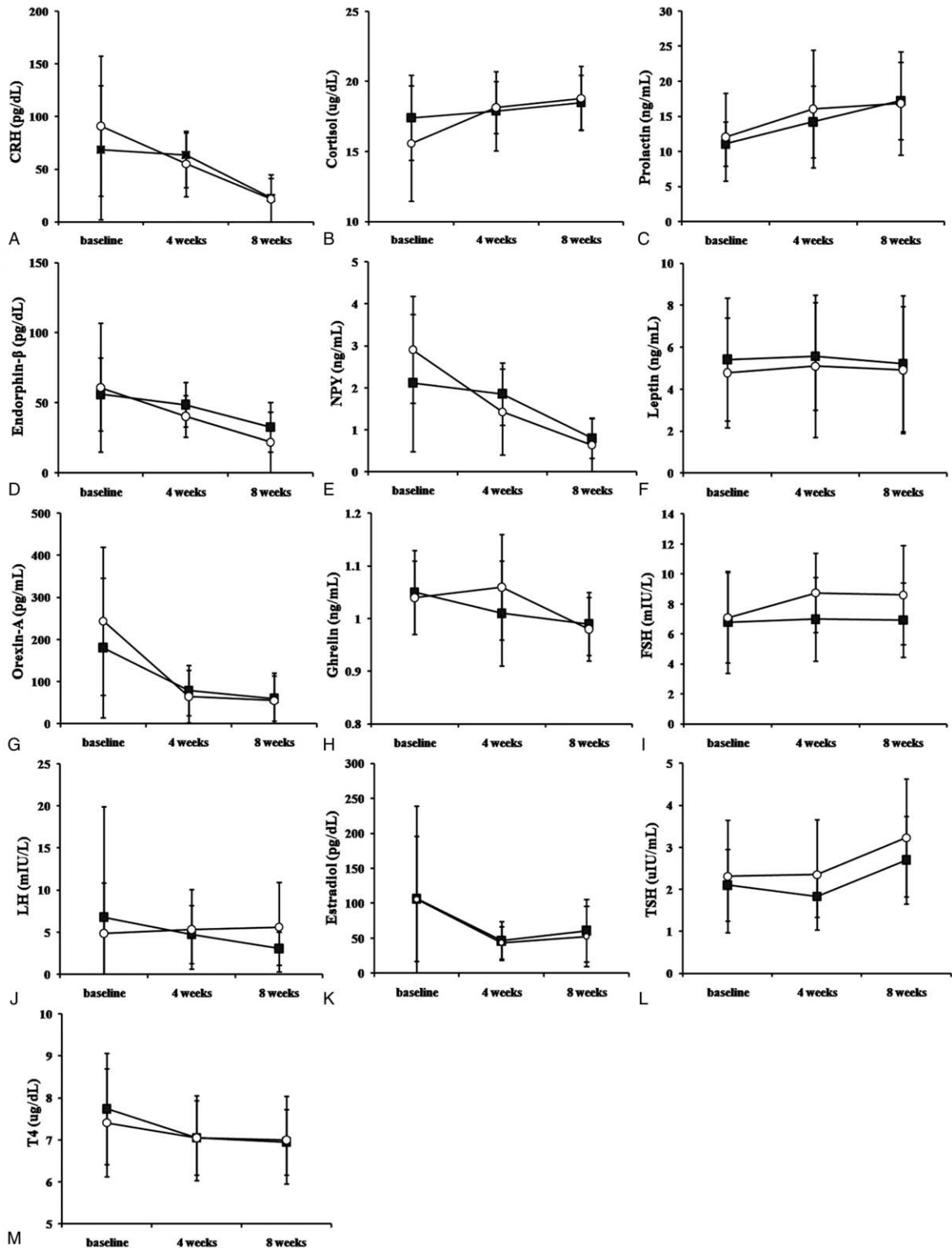
Figure 1. Levels of hormones and peptides during the 8-week training course for participants with regular menstruation (■) and those with irregular menstruation (○). There was no difference in levels of hormones and peptides between groups (repeated-measures analysis of variance [ANOVA]).

hormones and peptides according to menstrual regularity. The patterns of changes in hormones and peptides were similar between the 2 groups, and there was no difference in these levels between the 2 groups (repeated measures ANOVA).

4. Discussion

The changes in orexigenic peptides related to reproductive controls were evaluated in this study. NPY is concentrated in the hypothalamus, which regulates appetite and feeding behavior.[13] NPY regulates gonadotropin-releasing hormone (GnRH)-induced LH production and inhibits GnRH secretion in hypo-estrogenic conditions.[14] In humans, it has been shown that NPY is released in response to sympathetic activation by a number of stimuli, including hypoglycemia, exercise, and acute stress.[15] Ghrelin, which is released from the stomach, stimulates appetite, reduces fat utilization, and induces adiposity, as well as regulates the hypothalamus-pituitary-gonad axis. In humans, administration of ghrelin causes decreased gonadotropin pulsatility[16] and ghrelin levels are increased in adult athletes after exercise[17] and in nonathletes with anorexia nervosa.[18] Orexin-A also regulates appetite and food intake and has an inhibitory effect on LH secretion by influencing GnRH release.

On the basis of these previous reports, we hypothesized that orexin-A, ghrelin, and NPY levels would increase according to study period and be associated with menstrual irregularity. Thus, it may be of interest to note that the levels of these orexigenic peptides decreased, and there were no differences in levels between women with normal menstruation and those with abnormal menstruation, indicating little or no association of these peptides with menstrual irregularity in this study. The reason for this difference is unclear, but there is a plausible explanation. Exercise itself may influence orexigenic peptides. Pil-Byung et al[19] reported that ghrelin levels were significantly decreased by exercise after 8 weeks, whereas the levels showed a tendency to increase in controls, demonstrating the beneficial effects of an exercise program by altering appetite-regulating hormones. Otherwise, the levels of ghrelin and NPY may increase because of various conditions associated with inadequate feeding patterns and negative energy balance as part of the compensatory response.[20,21] This means that these peptides probably change because of the negative energy balance and not because of the exercise per se.[22] Moreover, a large body of evidence suggests that the mechanism responsible for menstrual disturbances in exercising women is consistent with an underlying energy deficiency. De Souza et al[23] also reported that ghrelin levels increased in the subjects with exercising associated amenorrhea, which may be due to chronic energy deficiency. Indeed, it is well documented that in exercising women, the failure to compensate dietary intake for the energy cost of exercise can have a profound suppressive effect on the reproductive axis.[24,25] Although exact volume and intensity of training could not be measured in this study, all participants completed all of physical training. Therefore, there was no difference in volume and intensity of training between the 2 groups. Moreover, participants were provided with 3300 kcal/day (carbohydrate 537 g, protein 123 g, and fat 73 g) throughout study period. Therefore, in this study, the association of orexigenic peptides with exercise may not be due to difference in degrees of energy balance between 2 groups although the exact caloric consumption was not measured. Another reason for this difference can be attributed to factors related to the study designs implemented, the participants enrolled, and the methods utilized. Other studies measured

orexigenic peptide levels before and after exercise and found increased levels after exercise.[17] Otherwise, in this study, orexigenic peptide levels were measured only before training at 4-week intervals and were compared. Moreover, orexigenic peptides were measured only once per day. Orexigenic peptide levels such as ghrelin have diurnal variations[26] and thus, the timing and frequency of sampling can give significantly different results and increase intra- and intervariability. In this study, however, to minimize diurnal variations, fasting blood sample was drawn at same time of the day (6:00–7:00 AM) after an overnight fast. Further studies with more frequent sampling per day considering the training schedules are needed to strengthen the result.

The hypothalamic-pituitary-adrenal (HPA) axis exerts profound, multilevel inhibitory effects on the female reproductive system. First, cortisol suppresses pituitary LH and ovarian estrogen and progesterone secretion, and renders target tissues resistant to E_2. Therefore, elevated cortisol levels are known to be responsible for menstrual disturbance caused by stress, as observed in persons with anxiety, depression, malnutrition, eating disorders, and chronic exercise, and in those with the hypogonadism of the Cushing syndrome.[27] Similarly, the cortisol level increased according to period in this study.

Physical or mental stress activates an instant increase of CRH, the principal central nervous system regulator. Luger et al[28] reported that an elevated resting cortisol level during intense training was involved in the hypersecretion of CRH in the hypothalamus. Indeed, the chronic increase in hypothalamic CRH content by running training has been reported in rats.[29] Therefore, increased CRH causes menstrual disturbance through activation of cortisol. Moreover, CRH has been known to produce a dose–dependent decrease in GnRH release by the hypothalamus in vitro and to reduce LH levels by the intraventricular administration of CRH in rats,[30] indicating that increased CRH directly causes menstrual disturbance. Therefore, we hypothesized a priori that CRH level as well as cortisol level would increase. Interestingly, the findings in this study suggest the opposite effect. The reason underlying this discrepancy from our results still remains unknown, although it might be caused by exercise-induced stress conditions. The HPA axis is regulated by arginine-vasopressin (AVP) and CRH, which are colocalized in the paraventricular nucleus of the hypothalamus. In acute stress, CRH and AVP synergistically stimulate pituitary adrenocorticotropic hormone secretion and, subsequently, cortisol secretion by the adrenal cortex. In contrast to acute stress, in response to chronic stress, there was a paradoxical reduction in CRH[31] and marked increase in AVP,[32] suggesting that the central activation of HPA activity has been taken over by a predominant AVP rather than the CRH drive.[33] Although it is difficult to differentiate chronic stress from acute stress, the activation of HPA activity and, in consequence, the release of cortisol might be caused by a predominant AVP rather than the CRH drive in this study. However, as AVP levels were not measured, further studies are certainly warranted.

Endorphin-β, the biochemical basis of "runner's high," has been known to be released by exercise.[34] As endogenous opiates inhibit gonadotropin secretion by suppressing hypothalamic GnRH,[35] we hypothesized that endorphin would increase according to study period and be associated with menstrual irregularity. However, endorphin levels decreased according to study period. Moreover, there was no difference in levels between the 2 groups.

In this study, prolactin levels increased according to study period, in line with other studies.[36] However, there was no

difference in prolactin levels between the 2 groups, similar to a study reporting that insignificant differences in prolactin were found when comparing eumenorrheic athletes with amenorrheic atheletes.[37] These results suggest that a mild increase in prolactin does not cause the suppression of the menstrual cycle.[38]

During study period, 4 participants were amenorrheic. We categorized participants into 3 groups according to their menstrual status during the training course as follows: regularly menstruating group (n=12), irregular but menstruating group (n=24), and amenorrhea group (n=4). On repeated measures ANOVA, levels of hormones and peptides were not different between the 3 groups (data not shown).

There is good evidence that exercise reduced E_2 levels[39–41] and these changes are reflective of a 2nd gonadotropin abnormality that may act in concert with the disruption of LH pulsatility to impair ovarian function, ovarian steroidogenesis.[39,40] Similarly, in this study, E_2 levels decreased throughout training period.

BMI and WC decreased according to period in this study, representing a desirable effect of exercise on physical fitness and body composition.

One of the limitations of this study was that we did not measure levels of hormones and peptides by the date of menstrual cycle but by the training schedule, at baseline, at 4 and 8 weeks after entering the study. Levels of certain hormones and peptides are affected by the date of menstrual cycle measured. Thus, these levels might not be comparable between the participants because the date of menstrual cycle at measurement varied. However, it was difficult to schedule the training courses individually by considering the menstrual cycles of the participants due to the fact that it is a military academy training course carried out as a group. These results therefore need to be interpreted with caution. We redid statistical analysis to minimize the effect of the date of menstrual cycle measured. Changes in levels of hormones and peptides during the 8-week training course between participants with regular menstruation and those with irregular menstruation over the 3 time points were assessed using general linear model with unstructured covariance structure. The date of menstrual cycle at baseline was included in the model as covariates to adjust for its difference between 2 groups. General linear model showed no difference in levels of hormones and peptides between 2 groups in line with results by repeated measures ANOVA (data not shown).

Another limitation was that there was no measure of central hormonal level; only peripheral hormonal level was measured. For example, Wang et al[42] demonstrated that plasma changes do not necessarily mirror NPY changes in the hypothalamus after 8 weeks of intensive training in rats. Moreover, psychological stress is believed to play an important role in causing menstrual irregularity. It has been found that different sources of stress, such as changes in occupation, stressful jobs, and school examinations, have a negative effect on the menstrual cycle.[43,44] Moreover, athletic competition was also found to cause menstrual irregularity without change in either body weight or body fat percentage.[45] In this study, psychological stress, such as that arising from competition, might have an influence on menstrual irregularity. Further studies are needed to validate the effects of such factors on menstrual irregularity.

The strength of this study lies in the selection of participants, who were all of reproductive age, had regular menstruation before the training, and had no hormonal treatment including OC. Moreover, the possible confounding factors, including exercise status, daily calories, and sleep duration, were controlled.

In conclusion, cortisol and prolactin levels increased but levels of CRH, endorphin-β, NYP, orexin-A, and ghrelin decreased throughout the training course. Moreover, the levels were not different between athletic trainees with normal menstruation and those with irregular menstruation.

Further research should extend these findings by investigating the exact mechanism by which high exercise levels, including intensive training, interfere with regular menstruation.

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