Effects of Menstrual Cycle Phase on Fluid Regulation during Walking Exercise

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Abstract

To elucidate the fluid regulation in different menstrual cycle phases during **e**xercise. Sex hormones affect fluid regulation in different ways. Moreover, the renin angiotensin-aldosterone system is activated in the luteal phase in rest. However, there are limited studies on fluid regulation affected by such hormone excretion in the menstrual cycle during exercise, especially during a light walking exercise. A non-invasive method using urine samples to determine menstrual cycle phases was used, and the follicular and luteal phases were successfully confirmed in 10 participants (age, 21 ± 1 years; body mass index, 20.5 ± 2.1 kg/m²). The experimental exercise sessions consisted of 5-min standing and 15-min walking at 2 km/h on 15% slope (approximately 8.3°) on a treadmill. Each participant carried a backpack weighing 5% of her own weight, and performed three sessions of walking exercise. Urine aldosterone excretion was significantly higher in the luteal than in the follicular phase before and after walking ($p <$ 0.05). Urinary excretion of aldosterone was five times higher in the luteal than in the follicular phase before and after walking exercise. Heart rates during walking, after rest, and after recovery were all significantly higher in the luteal than in the follicular phase (p < 0.05). The participants' ratings of perceived exertion during the first and third session of walking in the luteal phase was not higher than that at the follicular phase. The results of our study suggested that increased activity of the renin-angiotensin-aldosterone system in the luteal phase of the menstrual cycle might be further activated during exercise. This may increase the circulatory load, which is reflected as increased heart rate. These results suggested that premenopausal women may better take into account a possibility of an increased circulatory load in the luteal phase even when they perform light exercise.

Key words: Menstrual cycle, exercise, luteal phase, heart rate.

Introduction

Fluid regulation in different phases of menstrual cycle in women (Blum et al., 2014; Sawai et al., 2018), especially during exercise, has not been fully elucidated. In the luteal phase of the menstrual cycle, increased secretion of progesterone, results in elevation of the core body temperature by 0.2–0.5°C (Hayashi et al., 2012; Kolka and Stephenson, 1997; Lei et al., 2017). Such elevation of the body temperature in the luteal phase may cause increased sweating during exercise. The results of previous studies, however, are inconsistent regarding whether the sweat volume during exercise in the luteal phase increases or not. Some studies reported increases in the sweat volume during exercise in the luteal phase (Janse et al., 2012; Lee et al., 2014; Garcia et al., 2006), but the opposite has also been reported

(Kuwahara et al., 2005). However, several studies reported no change in the sweat volume irrespective of the menstrual cycle phase when performing an exercise (Janse et al., 2012; Lei et al., 2017). Sex hormones affect fluid regulation in different ways. Estrogen induces secretion of arginine vasopressin (AVP); therefore, AVP secretion varies during the menstrual cycle, depending on the estrogen secretion (Stachenfeld et al., 2001; Stachenfeld, 2014). The renin-angiotensin-aldosterone system (RAA) is activated in the luteal phase; therefore, it is possible to cause body fluid accumulation (Rosenfeld et al., 2008; Janowsky et al., 1973). Studies have also reported that sodium and water reabsorption is enhanced when estrogen and progesterone are presented together (Stachenfeld and Taylor, 2005; Stachenfeld, 2008). Further, studies on fluid regulation that have been affected by such hormone excretion in the menstrual cycle during exercise have been scarcely reported.

Previous studies investigating fluid regulation during the menstrual cycle utilized the blood levels of sex hormones for the determination of the follicular and luteal phases of the menstrual cycle, and in some cases, hormone administration was used (Stachenfeld, 2014). To elucidate fluid regulation in different menstrual cycle phases during exercise, we adopted a non-invasive method using urine samples to determine the phases of the menstrual cycle.

In our study, the participants performed walking exercise, which simulated hiking. Hiking has gained increased popularity and is considered a cost-effective exercise activity, which is beneficial for health (Mitten et al., 2018). Nevertheless, very few studies have evaluated the effect of menstrual cycle on body fluid regulation during walking on a slope while carrying a backpack, such as hiking. According to a reported work, women may present greater impact acceleration compared to men when walking with backpack (Lucas-Cuevas et al., 2013). We adopted 15% slope to simulate hiking and it was reported that energy cost and ventilation increase significantly at 15% than at 0% slope grade (Stephane and Nicolas, 2008). We used intermittent exercise to avoid overwork and dehydration because the participants were not familiar with exercise. After a total of 45 min walk in our experiment, the height reaches 217 m.

Here, we report that the aldosterone excretion was increased in the luteal than in the follicular phase during non-exercise condition and it was further increased after walking exercise. Such hormone changes may have affected the circulatory load because the heart rate is significantly higher in the luteal than in the follicular phase during walking exercise. Our results may have implications for the

exercise intensity in the luteal phase. Further, the efficiency of the urine luteinizing hormone (LH) test as a marker for estimating menstrual phase was highlighted, as this test could provide important information to women to adjust exercise intensity when being in the menstrual phase.

Methods

Participants

Fourteen healthy women (age range, 20–24 years) with regular menstrual cycles (Bull et al., 2019) voluntarily participated in this study. They were confirmed to have no cardiovascular and endocrine disease, and absence of using any medication that could interfere with the experimental procedure. Moreover, they did not have oral contraceptives for at least 1 year. None of the participants used to exercise (exercise more than 30 min, three times a week) and smoke. All of them provided written informed consent to participate. This study was approved by the Human Research Ethics Committee of our institution (no. 150007). In addition, this study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Procedure

The basal body temperature was measured with the basal thermometer (MC-52LC : OMRON HEALTHCARE Co., Ltd, Kyoto, Japan) in the supine position every morning for 2–3 months. The participants were instructed to measure their temperature under the tongue immediately after waking up before changing posture, and practiced how to measure the basal body temperature before participating in the experiment.

The day of ovulation was estimated by the detection of urinary LH using the Doe test LHa (ROHTO Pharmaceutical Co. Ltd., Osaka, Japan). Urine was tested every morning from the 10th day after the beginning of the last menstruation until positive results were obtained. In this study, the follicular phase of the menstrual cycle started from the 2nd day after the end of the last menstruation to 2 days before a positive urine LH test was detected and the luteal phase started from the 3rd day after the positive urine LH test, to 2 days before the beginning of the next menstruation.

The experimental sessions of walking exercise were performed by each participant in the follicular and luteal phases. The order of the first menstrual cycle phase was randomized for each participant and counterbalanced to minimize any habituation effects during the experiment (seven and seven participants started in the follicular and in the luteal phase, respectively; Figure 1).

Alcohol and caffeine intake were not allowed after

22:00 on the day before experiment. The participants were not allowed to perform rigorous exercise on the day before experiment and the experiment day. The experimental sessions started at 9:00 and ended at 16:00, and each participant was measured at the same hour of the follicular and luteal phase. Eating and drinking were not allowed from 2 h before the start of the experiment. Each participant urinated and, then, ingested 200 ml of water at 1 h before the experiment. The participants urinated again just before the start of the experiment, and the urine samples were collected for examination. After urination, the body weight was measured at 50 g accuracy using BC-309 (Tanita Corp., Tokyo, Japan) with the participants wearing only underwear to avoid possible influence of the weight of clothing on the measured weight. The experimental sessions comprised of 5 min of standing and, then, 15 min of walking at 2 km/h on 15% slope (approximately 8.3°) on a treadmill (MM5050SE: Fitness Apollo Japan Corporation, Tokyo, Japan), with each participant carried a backpack weighing 5% of her own weight (set in 100-g increments), which was the recommended weight from the perspective of posture and muscle activation (Yi-Lang and Ying-Cen,2018). Moreover, the walking speed was carefully selected prior to the study start by the research team to be safely measured.

After a 5-min rest, the same walking exercise was performed. Then, the participants took a 5-min rest, and the same walking exercise was performed again. In total, three sessions of walking exercise were completed by each individual. The participants were allowed to drink water freely during the rest periods. Urine was collected 30 min after the last walk. A pulse monitor (A300: Polar Japan, Tokyo, Japan) was attached to each participant's chest to monitor the heart rate every 1 min during the experiment. Blood pressure was measured with an automatic sphygmomanometer (CH-550: Citizen, Tokyo, Japan) that was placed on the participants' arms before the start of experiment and after each walking exercise.

The wet bulb globe temperature (WBGT), temperature, and humidity in the measurement room were adjusted with a humidifier and an air conditioner to keep them constant during the experiments, and they were monitored with a heatstroke index monitor (AD-5695: A & D Company, Tokyo, Japan).

An incremental 15-point Borg scale (Borg, 1970) was used to determine the participants' ratings of perceived exertion (RPE). RPE was measured before walking, every 1 min during walking, and after the end of walking. The visual analog scale (VAS) with a 100-mm line was used to evaluate fatigue and thirst: 0 mm corresponds to no perception and 100 mm to worst perception.
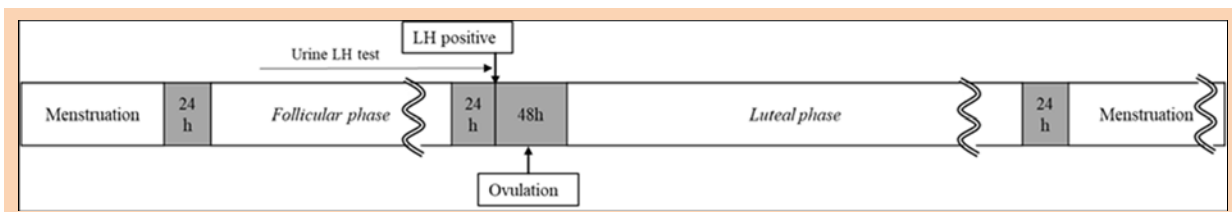
Figure 1. Estimation for menstrual phases. The periods in which the experiment was avoided are shaded in the figure.

The urine volume was measured immediately after urine collection, and urine pH was measured using *in vitro* diagnostic tools (Certification number 21500AMZ00525000: Samwa Kagaku Kenkyusho Co.,Ltd, Aichi, Japan) in our laboratory. Part of the urine was divided into two parts (refrigerated and frozen) and, then sent to the measurement laboratory (Falco biosystems Ltd, Kyoto, Tokyo). The refrigerated urine samples were used to measure urine osmolality (freezing point measurement method) and sodium content (electrode method). The frozen samples were used for the measurement of aldosterone levels (radioimmunoassay method).

Water intake volume was determined by measuring the weight of the water bottle using the cooking scale (KW-320: Tanita Corp., Tokyo, Japan) after each resting period. The sweat volume was estimated using the following formula:

Sweat volume (g) = Weight before walking (g) – Weight after walking (g) + Water intake volume (g)

Statistical analysis

The measurement data are expressed as means ± standard deviation. Analyses were performed with SPSS version 25 (IBM Corp., Armonk, NY, USA). The effect size calculations were performed using Microsoft Excel (Microsoft, Redmond, WA, USA).

The distribution of each variable was checked for normality using the Shapiro-Wilk test. When the data were normally distributed (basal body temperature, WBGT, temperature, humidity, sweat volume, water intake, body weight, urine volume, urine pH, urine osmolarity and urine sodium excretion), a paired sample t test was used. In the case where the data were not normally distributed (BMI and urine aldosterone), the Wilcoxon signed rank test was used. Two-way analysis of variance (ANOVA) for repeated measures (phase × time) was used to compare the heart rate, blood pressure, RPE, and perception of fatigue and thirst. The level of statistical significance was set at $p < 0.05$ or $p < 0.01$, and the effect size was added when significant difference was found. The effect size of t test and the Wilcoxon signed rank test was calculated using r changing Cohen's D, while the corresponding of two-way ANOVA for repeated was calculated using η2 (Cohen, 1988 ; Lakens, 2013). A post hoc power analysis was performed using G* power 3.1.7.9 (Heine-Universität, Düsseldorf, Germany).

Results

For one participant, no positive urine LH result was obtained until the next menstruation cycle, and the basal body temperature did not show two phases. One participant retracted the consent after the measurement in one phase. For two participants, walking experiment was performed within 24 h of the positive urine LH test or the start of menstruation. Thus, the results of the walking exercise from these four participants were not used. Six and four participants were first measured in the follicular and the luteal phase, respectively.

The basal body temperature was elevated in each participant on the days after the positive LH urine test (compared to pre-test measurements 36.30 ± 0.39°C vs. 36.74 ± 0.38°C; $p < 0.01$; $r = 0.81$), suggesting that the positive test corresponded to ovulation. Therefore, it was shown that the urine LH test could be used for estimation of the follicular and luteal phases of the menstrual cycle. The demographic characteristics of these participants are shown in Table 1.

Table 1. Demographic characteristics of the participants (n = 10), sweat volume, and water intake during walking in the follicular and luteal phases. Data are means ± standard deviation.

	Follicular phase	Luteal phase	p value
Age (years)	21 ± 1 (20 - 25)		
Height (m)	1.615 ± 0.08 (1.51 – 1.72)		
BMI (kg/m²)	20.5 ± 2.1	20.5 ± 2.2	0.476
Sweat volume (g)	234 ± 52	241 ± 45	0.563
Water intake (g)	388 ± 120	356 ± 96	0.362

The BMI of the participants ranged in the follicular and the luteal phases from 18.7 kg/m² to 25.8 kg/m², and from 18.7 kg/m² to 26.1 kg/m², respectively. BMI, body mass index.

The environmental conditions were not significantly different between the follicular and luteal phases. Room temperature, humidity, and WBGT readings in the follicular and luteal phases were 26.4 ± 1.7°C and 26.5 ± 0.9°C ($p = 0.891$), 51.2 ± 6.0% and 50.7 ± 4.1% ($p = 0.871$), and 21.9 ± 0.9°C and 21.4 ± 0.8°C ($p = 0.250$), respectively.

Body weight significantly increased after walking in the follicular and luteal phases respectively ($p < 0.01$) (Table 2). No significant difference was found in the body weight between the follicular and luteal phases. Sweat and water intake volumes during exercise did not differ significantly between the follicular and luteal phases (Table 1). Urine volume was larger after than before walking, but the difference was significant only for the follicular phase ($p < 0.05$) (Table 2). Urine volume before walking was significantly larger in the luteal than in the follicular phase ($p < 0.01$) (Table 2). Urine pH before walking did not differ significantly from that after walking in both follicular and luteal phases (Table 2). Urine sodium excretion was higher in the luteal than in the follicular phase before and after walking, but the difference was statistically significant only before walking ($p < 0.05$) (Table 2).

Urine aldosterone excretion was significantly higher in the luteal than in the follicular phase before and after walking respectively ($p < 0.05$, $r = 0.512$; $p < 0.05$, $r = 0.558$) (Figure 2). Aldosterone excretion significantly increased after walking in the follicular and luteal phases ($p < 0.01$, $r = 0.604$; $p < 0.01$, $r = 0.627$) (Figure 2).

Heart rates during walking, after rest, and after recovery (30 min after walking) were all significantly higher in the luteal than in the follicular phase ($F = 15.73$, $p < 0.01$, $η^2 = 0.052$; $F = 17.36$, $p < 0.01$, $η^2 = 0.057$; $F = 11.85$, $p < 0.01$, $η^2 = 0.040$, respectively) during walking, after rest, and after recovery, respectively, two-way repeated measures ANOVA), and no association was detected between the menstrual cycle phase and time (Figure 3). A post hoc power analysis was performed for the analyses of

Table 2. Body weight, urine volume, and chemical properties before and after walking in the follicular and the luteal phases. The data are means ± standard deviation (n = 10).

	Follicular phase			Luteal phase			Follicular phase vs Luteal phase	
	Before walking	**After Walking**	**Before vs After p Value (effect size)**	**Before walking**	**After walking**	**Before vs After p Value (effect size)**	**Before p Value (effect size)**	**After p Value (effect size)**
Body weight	52.80 ± 6.01	52.95 ± 6.01‡	0.003 (0.81)	52.91 ± 6.28	53.02 ± 6.25‡	0.003 (0.80)	0.687 (0.14)	0.795 (0.09)
Urine volume (ml/h)	25.6 ± 14.6	67.2 ± 55.1‡	0.017 (0.70)	59.0 ± 24.1 †	62.3 ± 36.7	0.148 (0.47)	0.002 (0.83)	0.753 (0.11)
Urine pH	6.0 ± 1.1	5.9 ± 0.7	0.662 (0.15)	6.6 ± 1.0	6.0 ± 0.7	0.111 (0.51)	0.119 (0.50)	0.662 (0.15)
Urine osmolarity (mOsm/L)	894 ± 155	737 ± 303	0.130 (0.49)	730 ± 155 †	715 ± 217	0.782 (0.09)	0.047 (0.61)	0.822 (0.08)
Urine sodium excretion(mEq/h)	3.9 ± 2.2	4.8 ± 3.2	0.403 (0.28)	9.3 ± 5.7 †	6.5 ± 4.8	0.177 (0.44)	0.019 (0.69)	0.240 (0.39)

† $p < 0.05$ between follicular and luteal phases. ‡ $p < 0.05$ between before and after walking.
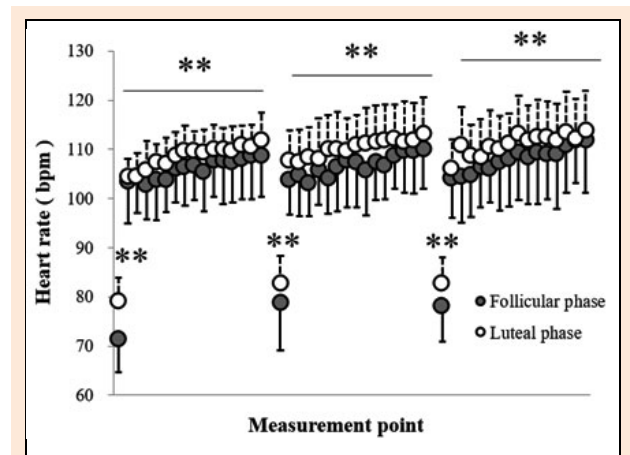
urine aldosterone and heart rate and the results showed that these analyses were sufficiently powered (i.e. the power was larger than 80% at the significance level of <0.05 for each analysis).
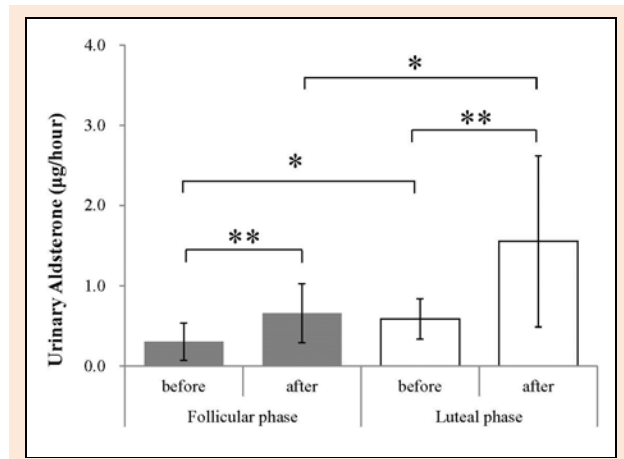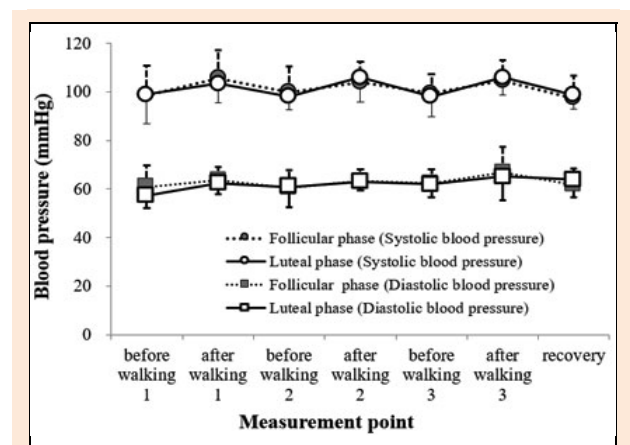

Figure 2. Urine aldosterone excretion before and after walking in the luteal and follicular phases. The values are presented as means ± standard deviation (n = 10). * p < 0.05, ** p < 0.01.

Figure 3. Heart rates during walking and after rest in the follicular and luteal phases. The values are presented as means ± standard deviation (n = 10). bpm, beats per min. ** p < 0.01. Three walking sessions, each lasting 15 min, were performed with a 5-min rest between them. Heart rate was measured every 1 min during walking and after each rest period. The heart rates during walking, after rest, and after recovery (30 min after the third walking session) in the luteal phase were all significantly higher than the values in the follicular phase, as revealed by the two-way ANOVA for repeated measures.

Neither systolic nor diastolic blood pressure differed significantly between the follicular and luteal phases, and no interaction was detected between the phase and time effects in the systolic and diastolic blood pressures respectively (p = 0.978, p = 0.578) (Figure 4).

Although RPE during the first and third walking sessions did not differ significantly between the follicular and luteal phases, RPE during the second walking session was significantly higher in the luteal than the corresponding in the follicular phase (p = 0.626, F = 4.461, p < 0.05 and $\eta^2 = 0.013$, p = 0.610) (Figure 5). No association was detected between the menstrual cycle phases and time effects during walking. No significant difference was observed between the perceptions of thirst (100-mm VAS) in the follicular and luteal phases (p = 0.097) (Figure 6).
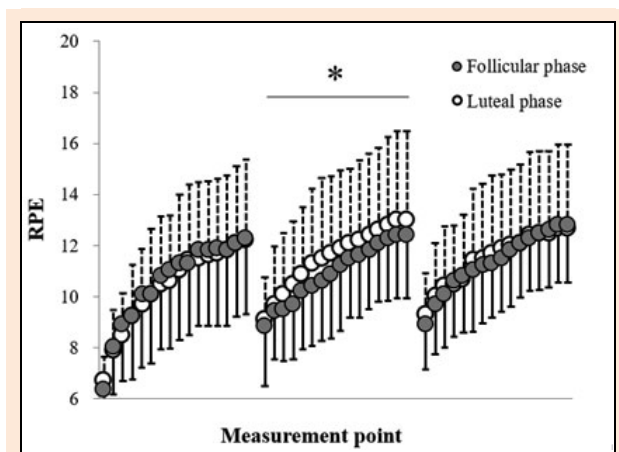

Figure 4. Systolic and diastolic blood pressures before and after walking. The values are presented as means ± standard deviation (n = 10). Three walking sessions, each lasting 15 min, were completed with 5-min rest between walking sessions. Blood pressure did not differ between the follicular and luteal phases.

Figure 5. RPE during walking in the follicular and luteal phases. The values are presented as means ± standard deviation (n = 10). Three walking sessions, each lasting 15 min, were completed with 5-min rest between walking sessions. RPE during the second walking session in the luteal phase was significantly higher than that in the follicular phase, as analyzed by the two-way ANOVA for repeated measures. RPE, ratings of perceived exertion. * $p < 0.05$.
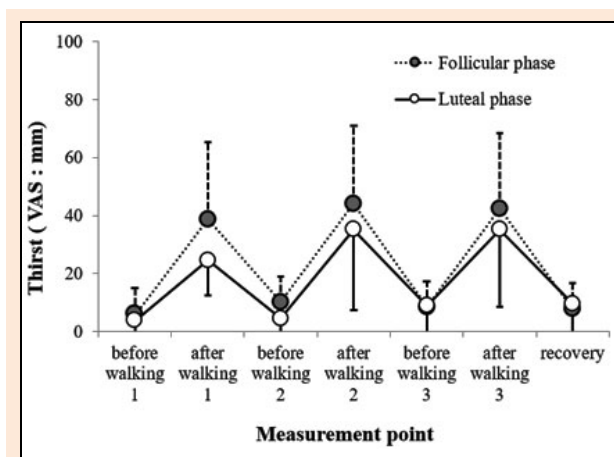
Figure 6. Feeling of thirst (as measured by 100-mm VAS) during walking. The values are presented as means ± standard deviation (n = 10). No significant difference was observed. VAS, visual analog scale.

Discussion

This study evaluated the circulatory and hormonal changes that occur with respect to fluid regulation in the luteal and follicular phases of the menstrual cycle during walking exercise that simulates mountain hike. Under resting conditions, the plasma concentration and urinary excretion of aldosterone have been reported to be higher in the luteal than in the follicular phase (Castagna et al., 2011; Davis et al., 2017; Stachenfeld et al., 1999; Szmuilowicz et al., 2006). This study obtained similar results for urine. Further, we showed that urinary excretion of aldosterone increased after walking exercise that simulated mountain hike in the follicular and luteal phases. Urinary excretion of aldosterone after exercise in the luteal phase was five times higher than the value before walking in the follicular phase, suggesting that the fluid load on the circulatory system is increased in the luteal phase especially during exercise. This may be supported by the present observation that the heart rate during exercise was higher in the luteal than in the follicular phase. Although the present findings were

obtained under the condition of free water intake, increased heart rate during walking exercise with water intake restriction has been reported (Nose et al., 2017). Previous studies have also reported higher heart rates during rest and exercise in the luteal than in the follicular phase (Balayssac-Siransy et al., 2014; Barba-Moreno et al., 2019; Fukuoka et al., 2002; Gordon and Girdler, 2014). In contrast, some researchers (Hackney et al., 2019; Hayashi et al., 2012; Kuwahara et al., 2005; Vaiksaar et al., 2011) reported that the heart rate did not differ between the follicular and luteal phases.

Although exposure to estrogen and progesterone has been reported to result in increased circulating plasma volume (Stachenfeld and Taylor, 2005), its effect on overall body water balance was minimal in healthy young women (Stachenfeld, 2008; Wenner and Stachenfeld, 2012). Therefore, the increased heart rate in the luteal phase could not be attributed solely to the plasma volume increase. Elevation of the body temperature in the luteal phase may also be related to the increase in the heart rate in the luteal phase.

Blood pressure was not higher during rest or exercise in the luteal than in the follicular phase, although increased secretion of aldosterone in the luteal phase, especially after exercise, was observed. As systemic vascular resistance is lower in the luteal than in the follicular phase (Gordon and Girdler, 2014), effects of RAA on blood pressure may have decreased. Likewise, previous studies reported no change in blood pressure in the luteal phase despite the increased aldosterone levels in blood and urine (Szmuilowicz et al., 2006; Davis et al., 2017; Fu et al., 2010). Our results showed no elevation in blood pressure from the resting value during exercise in the luteal phase.

A higher level of RPE, which is a subjective index of exertion, was observed in the luteal compared with the corresponding in the follicular phase only in the second session of walking exercise. Interestingly, higher RPE levels in the luteal phase have been previously reported (Janse et al., 2012; Pivarnik et al., 1992). RPE is affected by the increased heart rate and body temperature during aerobic exercise (Willmott et al., 2018). The reason for the higher RPE only during the second session of walking in this study is not clear, as the heart rate in the second session did not differ from the corresponding in the first and third sessions of walking.

We speculated that the urine volume in the luteal phase would decrease due to RAA activity. However, a previous study (Fong and Kretsch, 1993) reported that the urine volume was higher in the luteal than in the follicular phase due to dietary factors. Here, there was no strict control of food content and volume intake on the night before the experiment. Thus, it is possible that the urine volume was higher in luteal than in the follicular phase before walking because of meal content. Urine volume after walking was not significantly different between the two phases. Our results did not clarify the relationship between renin activity and urine volume.

Although it was expected that sweat volume would be higher in the luteal than in the follicular phase during walking because of the higher body temperature in this phase, no significant difference in sweat volume during

exercise between follicular and luteal phases was observed. Previous studies have reported conflicting results regarding sweat volume during exercise in the luteal phase (Garcia et al., 2006; Janse et al., 2012; Kuwahara et al., 2005; Lee et al., 2014; Lei et al., 2017). A study reported no difference in the water volume taken throughout the menstrual cycle (Fong and Kretsch, 1993). As estrogens suppress thirst (Somponpum, 2007), we did not perform any experiment during the 2 days before and after ovulation, when estrogen excretion rapidly increased. In our study, this may be related to the finding that there was no difference in the level of thirst and the volume of water taken between the luteal and follicular phases. Moreover, low exercise intensity, such as 1-h walking, may not have affected the fluid balance in the luteal or in the follicular phase.

This study had several limitations. First, the direct RAA activation and the effects of sex hormones on RAA could not be evaluated because blood levels of renin, aldosterone, and sex hormones were not measured. Therefore, urine aldosterone levels may have been affected by the urine volume, especially in the comparison of the aldosterone levels between follicular and luteal phases before exercise because urine volume was significantly higher in the luteal phase than in the follicular phase before exercise. Second, as the participants were young women aged 20–21 years, our results may not be generalized to women of other ages. Third, the number of participants was limited, and only light-intensity exercise was performed in this study; we hypothesized that exercise of greater intensity, which would result in profuse sweating, could give different results. Finally, water intake and diet were not severely controlled before experiment. Lack of strict control of water intake and diet may have confounded the study results including urine volume and content. The study subjects, however, did not change their diet habits during the study period.

As circulatory load might be increased in the luteal phase, further studies that use different exercise intensities, taking into account the menstrual cycle phases are required. The urine LH test might be used as a marker to estimate the menstrual phase, and a useful tool for women to adjust exercise intensity.

Conclusion

To conclude increased RAA activation in the luteal phase of the menstrual cycle may be further induced during exercise. This may result to increased circulatory load, which is reflected as increased heart rate. However, such increases in the circulatory load may not be recognized by an individual.

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Key points

- Heart rates during walking, after rest, and after recovery were all significantly higher in the luteal than in the follicular phase.
- Activity of the renin-angiotensin-aldosterone system in luteal phase increase the circulatory load, which is reflected as increased heart rate.
- Increased activity of the renin-angiotensin-aldosterone system in the luteal phase of the menstrual cycle might be further activated during exercise.
- Our results suggested that premenopausal women may had better take into account a possibility of an increased circulatory load in the luteal phase even when they perform light exercise.

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