早稲田大学審査学位論文
博士（スポーツ科学）

Effects of menstrual cycle phase on thermoregulation and inflammatory response during prolonged exercise under hot conditions

異なる月経周期における暑熱環境下長時間運動中の体温調節および炎症反応に飲料摂取が及ぼす影響

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Abbreviations

LP: Low progesterone level phase

LP-non: non-ingestion trial during the LP

LP-wat: water ingestion trial during the LP

HR: Heart rate

HP: High progesterone level phase

HP-non: non-ingestion trial during the HP

HP-wat: water ingestion trial during the HP

RER: Respiratory exchange ratio

RPE: Ratings of perceived exertion

$\dot{V}E$: Minute ventilation

$\dot{V}CO_2$: Carbon dioxide production

$\dot{V}O_2$: Oxygen consumption

CHO: Carbohydrate

Tc: Core temperature

FA: Follicular phase placebo trial

FC: Follicular phase carbohydrate trial

LA: Luteal phase placebo trial

LC: Luteal phase carbohydrate trial

PRE: Before exercise

IL: Interleukin

MPO: Myeloperoxidase

TNF: Tumor necrosis factor

POST: High intensity time trial performance test

Preface

Premenopausal women experience menstrual cycles, which are accompanied by fluctuations in reproductive hormones. As many women now participate in sports activities for health promotion, weight management, social association, and personal reasons, the interest in the effect of the menstrual cycle phase on physiological responses during exercise has increased. As the menstrual cycle phase is known to have effects on physiological responses such as substrate metabolism, body temperature regulation, and endocrine response at rest, it is not difficult to consider the effect of the menstrual cycle phase on physiological responses during exercise. Investigating the physiological responses over several menstrual cycles during exercise may help to avoid the risk of injury and damage, or to improve health and exercise performance. In the present study, we set 2 challenges to investigate the effect of the menstrual cycle phase on physiological response during prolonged exercise under hot conditions. One is to investigate the influence of the menstrual cycle and water ingestion on physiological response, whereas the other is to assess the influence of the menstrual cycle and carbohydrate ingestion on inflammatory response.

Chapter1. The effect of the menstrual cycle and water ingestion on physiological responses during prolonged exercise under hot conditions

1. Background

In women with a normal menstrual cycle, core temperature changes periodically, increasing by 0.3°–0.5°C in the luteal phase as compared with the follicular phase [1]. Previous studies have suggested that progesterone and estradiol are involved in core temperature regulation in women [2,3]. Although details of the mechanism underlying thermoregulation via these hormones in humans remain unclear, these hormones are likely to exert their effect via the preoptic area of the brain [4]. Stephenson et al. suggested the possibility that core temperature fluctuations associated with the menstrual cycle is due to peripheral heat dissipation [5].

During exercise, core temperature thresholds for perspiration and skin vasodilatation are also influenced by the menstrual cycle phases as well as by resting core body temperature [6,7]. The study of oral contraceptive administration in women has suggested that progesterone is involved in the increase in the core temperature threshold for heat dissipation responses that are observed in the luteal phase and that

estradiol attenuates the effect of progesterone [3]. In addition, core temperature thresholds for perspiration in the late follicular phase are lower than those in the early follicular phase [8]. Thus, reproductive hormones modify thermoregulatory responses during exercise; it appears that estrogen decreases the thresholds for perspiration and skin vasodilatation; whereas, progesterone increases these thresholds. Although most studies reported that the menstrual cycle had little effect on the sensitivity of the heat dissipation reaction [3,6,8,9], Kuwahara et al. reported that the heat dissipation reaction was weaker in the luteal phase than in the follicular phase [10]. They suggested that the increase in progesterone levels in the luteal phase causes a decrease in plasma volume, which then leads to decreased heat dissipation via the baroreceptor reflex during exercise at moderate intensity and moderate temperature (25°C); however, the presence of high heat stress (35–50°C) and high exercise intensity (60–85% $\dot{V}O_2$ max) may have masked the effects of the menstrual cycle in the other studies.

With prolonged exercise under hot conditions, sweating decreases body fluid and leads to a rise in plasma osmolarity, which consequently hampers circulatory function [11-14]. Maintenance of body fluid levels by ingesting water during exercise

under hot conditions suppresses the increase in body temperature and the related decline in exercise performance [15]. Studies that have investigated the effects of the menstrual cycle phase on cardiorespiratory responses, thermoregulation, and endurance performance have typically been carried out either without including water ingestion [16-19] or with water ingestion (ad libitum or controlled) during the exercise [20,21]. To our knowledge, no studies have investigated the effects of both the menstrual cycle and water ingestion during exercise on physiological responses under hot conditions. Therefore, in the present study, we aimed to investigate the effect of the menstrual cycle and water ingestion on physiological responses during prolonged exercise at moderate intensity under hot conditions.

2. Material and methods

2.1. Subjects

This study was approved by the Human Research Ethics Committee of the Faculty of Sport Sciences of Waseda University for use of human subjects in accordance with the Declaration of Helsinki. Eight healthy young women (age: 22.9 [SD 2.2] years; height: 159.8 [SD 3.7] cm; weight: 50.9 [SD 6.2] kg; and $\dot{V}O_{2peak}$: 35.8 [SD 7.3] $ml \cdot min^{-1} \cdot kg^{-1}$)

with regular menstrual cycles volunteered to participate in this study. Six subjects had not performed regular physical activities for the previous three years, whereas two other subjects performed regular physical activities (softball and weightlifting).

2.2. Experimental design

Based on the progesterone levels, we divided the menstrual cycle into two phases—the low progesterone level phase (LP) and high progesterone phase (HP)—as progesterone levels were likely to be related to the increase in body temperature. This study comprised four separate trials. Subjects performed two trials during the LP and two trials during the HP. In each menstrual cycle phase, one trial was performed without water ingestion (non-ingestion trial) and the other with water ingestion (water ingestion trial). The phase of the menstrual cycle was determined by the basal temperature, the day of menstruation and the serum progesterone level. We used a progesterone level of > 5.1 ng/mL as the threshold for defining the HP [22,23]. To avoid the confounding of phase effects by trial order, subjects were allocated randomly with different trial orders, with four subjects starting the trials during the LP and another four subjects starting the trials during the HP. However, the non-ingestion trial was performed before the water

ingestion trial because the amount of water ingested during trial was determined by the body mass loss experienced in the non-ingestion trial. Each trial was performed on a separate day, at least 1 week apart. We asked subjects to ingest nothing except water after 21:00 on the day before the experimental trial, and the subjects ate a standardized breakfast (protein: 12.4 g; fat: 5.5 g; carbohydrate: 75.7 g; total energy: 395 kcal) at 6:00, which was 6–7 h before the trial. After breakfast, no food or beverages, except for water, were allowed. Furthermore, we asked subjects to conduct their days similarly, including the type and amount of food ingested on the day before the trial.

2.3. Preliminary testing

To estimate the phase of the menstrual cycle, all subjects recorded their oral temperature upon waking every day for at least two months, as well as the day of menstruation for three months, before commencement of the trial. Additionally, estradiol and progesterone levels in blood samples taken before commencement of the exercise were analyzed to confirm the menstrual cycle phase.

$\dot{V}O_{2peak}$ was measured using a maximal graded exercise test, with an electromagnetically braked cycle ergometer (Combi RS-232; Combi, Tokyo, Japan).

The initial workload was 0 W for 4 min during warming up, and was increased by 30 W every 3 min thereafter, starting at 40 W, until subjects could no longer maintain the required pedaling frequency (70 rpm). Heart rate (HR) was monitored by electrocardiography (Cardiosuper 2E32; Sanei-Sokki, Yamagata, Japan) throughout the exercise. During the progressive exercise test, the gas expired by the subjects was collected, and the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured and averaged over 30-s intervals, using an automated breath-by-breath gas analyzer (Minato AE300; Minato Medical Science, Osaka, Japan). $\dot{V}O_{2peak}$ was defined as the highest 30-s value. At the end of each workload stage, subjects were asked to indicate the rating of perceived exertion (RPE) using the Borg scale [24]. The Borg scale consists of 15 grades from 6 to 20, with 7 = Very, very light, 9 = Very light, 11 = Fairly light, 13 = Somewhat hard, 15 = Hard, 17 = Very Hard, 19 = Very, very hard.

2.4. Experimental trials

To minimize the potential effects of acclimatization, experiments were conducted from February to early May. In all trials, subjects performed cycling exercise at 70 rpm,

corresponding to a workload-estimated 50% $\dot{V}O_{2peak,}$ for 90 min under hot conditions (30 [SD 2] °C, 50 [SD 5] % relative humidity). The workload corresponding to 50% $\dot{V}O_{2peak}$ was determined from a graded exercise test by interpolation from the line of best fit describing the relationship between power output and $\dot{V}O_{2peak}$. Each subject performed a familiarization trial using the same exercise protocol as the experimental protocol, more than one week before the experimental trial. To measure rectal temperature during exercise, each subject inserted a rectal probe herself (401J; Nikkeiso-YSI Co., Ltd., Musashino, Japan) to 10 cm past the anal sphincter. During exercise, minute ventilation ($\dot{V}E$), respiratory exchange ratio (RER), HR, and rectal temperature were measured for 3 min each during the 4-min warm-up, and again at 15 min, 30 min, 45 min, 60 min, 75 min, and 90 min time points. Subjects were asked to indicate their overall RPE, cardiovascular RPE, and leg RPE, to identify specific locations of perceived exertion, at every 15-min time point from the warm-up to the end of the 90-min cycling exercise.

During the water ingestion trial, subjects ingested water every 15 min (for a total of 7 times) during exercise, and the total amount of water ingested equaled the body

weight loss determined during the non-ingestion trial. The temperature of the water used was 5 [SD 2] °C; this temperature was chosen because of the high palatability of such water, which is known to influence fluid consumption and help prevent heat illness during endurance exercise [25].

2.5. Blood variables and reproductive hormone level analysis

Venous blood samples were collected by venipuncture from an antecubital vein before and after exercise. Blood samples were collected into serum separation tubes or vacutainers containing ethylenediaminetetraacetic acid (EDTA). A fraction of whole blood was used to measure hemoglobin and hematocrit. The blood was allowed to clot in the serum separation tubes at room temperature for 30 min, while vacutainers containing EDTA for plasma separation were immediately centrifuged at 1,000× g for 10 min. Serum and plasma were then removed and stored at -80ºC for subsequent analysis. The serum concentrations of Na, osmolarity, hemoglobin, and hematocrit were measured. The serum concentrations of estradiol and progesterone were measured using an anti-thyroglobulin antibody (ECLIA). The reference values of estradiol levels for non-pregnant premenopausal women, using this technique, are 20–85 pg/ml in the early

follicular phase, 25–350 pg/ml in the late follicular phase, 50–550 pg/ml in the ovulation period, and 45–300 pg/ml in the luteal phase. The reference values of progesterone levels for non-pregnant premenopausal women, using this technique, are <0.92 ng/ml in the follicular phase, <2.36 ng/ml during the ovulation period, and 1.28–29.6 ng/ml in the luteal phase. These blood parameters were analyzed by SRI, Inc (Tokyo, Japan).

3. Statistical analysis

All data were assessed for normal distribution using the Shapiro–Wilk statistic. Data for body weight, plasma osmotic pressure, serum sodium concentration, hemoglobin concentration, hematocrit, plasma volume change, rectal temperature, HR, $\dot{V}$E, and RER were normally distributed. The estradiol data that were normally distributed after log transformation, were analyzed using the paired *t*-test for each trial. Progesterone data that were not normally distributed, were analyzed using the Wilcoxon signed-rank test for each trial. The normally distributed data (body weight, plasma osmotic pressure, serum sodium concentration, hemoglobin concentration, hematocrit, and plasma volume change) were analyzed using a 2 × 2 factor (menstrual cycle phase × water condition)

repeated analysis of variance (ANOVA). When significant menstrual cycle phase effects, water condition effects, or interactions (menstrual cycle phase × water condition) were evident, Bonferroni posthoc multiple comparisons were used. The differences in body weight, plasma osmotic pressure, sodium concentration, hemoglobin concentration, hematocrit, and plasma volume change before and after exercise were analyzed using the paired *t*-test. The other normally distributed data (rectal temperature, HR, VE, and RER during exercise) were analyzed using a 4 × 7 factor (trial × time) repeated ANOVA. When significant trial effects or trial × time interactions were evident, we conducted further analysis using a 2 × 7 factor (menstrual cycle phase × time) repeated ANOVA in both water conditions and a 2 × 7 factor (water conditions × time) repeated ANOVA in both menstrual cycle phases. Data for overall RPE, cardiovascular RPE, and leg RPE, which were not normally distributed, were analyzed using the Kruskal-Wallis' one-way ANOVA to determine the trial effects at specific time-points. Analyses were performed using SPSS version 20 software for Windows (IBM Corporation, Armonk, NY, USA), with the threshold for statistical significance set at $P = 0.05$. The relationships between the dependent variables and the serum reproductive hormone levels were assessed by establishing Pearson product-moment correlation coefficients, with the level of

statistical significance set at $P = 0.05$.

4. Results

4.1. Reproductive hormones

Table 1-1 shows the serum estradiol and progesterone levels of the subjects. All subjects exceeded the 5.1 ng/ml progesterone level set for the HP in both trials. Because the serum progesterone level of subject No. 8 in the non-ingestion trial was out of range for the LP, we excluded this subject and analyzed the remaining 7 subjects. Estradiol levels during the HP were significantly higher than those during the LP only in the non-ingestion trial ($P < 0.05$). Progesterone levels were significantly higher during the HP than during the LP in each trial ($P < 0.001$).

Table 1-1. Individual estradiol and progesterone levels of all subjects

	Non-ingestion trial				Water ingestion trial			
	Estradiol (pg/ml)		Progesterone (ng/ml)		Estradiol (pg/ml)		Progesterone (ng/ml)	
Subject No	LP	HP	LP	HP	LP	HP	LP	HP
1	41.0	227.0	0.57	7.10	57.0	374.0	0.56	7.80
2	45.0	66.0	0.45	5.70	67.0	264.0	0.54	6.10
3	59.0	49.0	2.18	5.20	122.0	99.0	0.61	8.20
4	116.0	185.0	0.25	5.40	288.0	263.0	0.52	6.20
5	13.0	51.0	0.57	8.20	13.0	212.0	0.57	7.10
6	26.2	122.0	0.80	5.20	225.0	108.9	1.10	6.40
7	50.6	208.0	0.20	7.30	47.2	86.7	0.10	10.10
8	51.0	46.9	4.10	9.80	242.2	151.2	0.40	16.40
Median	45.0	122.0*	0.6	5.7**	67.0	212.0	0.6	7.1**
QD	10.6	69.0	0.2	0.9	60.7	79.8	0.03	0.9

LP, low progesterone level phase; HP, high progesterone level phase; QD, quartile deviation. Median and QD data for 7 subjects (excluding subject No. 8). *Significantly higher than the low progesterone level phase ($P < 0.05$), **Significantly higher than the low progesterone level phase ($P < 0.001$)

4.2. Body weight and blood variables before and after exercise

Body weight and blood variables before and after exercise are shown in Table 1-2. There was no significant difference in body weight before exercise between the menstrual cycle phases in each trial (Table 1-2). The body weight of the subjects were significantly decreased during 90 min of exercise in the non-ingestion trial in both menstrual cycle phases ($P < 0.01$), but were not different before and after exercise in the

water ingestion trial. The amount of sweat estimated from body weight loss and water ingestion during exercise was 0.92 [SD 0.38] kg during the HP and 0.77 [SD 0.22] kg during the LP during the non-ingestion trial, and 0.98 [SD 0.59] kg during the HP and 0.74 [SD 0.37] kg during the LP during the water ingestion trial. There was no difference between the menstrual cycle phases in each trial.

Plasma osmotic pressure was significantly increased during exercise in both menstrual cycle phases during the non-ingestion trial ($P < 0.05$), whereas plasma osmotic pressure during the water ingestion trial was not changed during the LP but significantly decreased during the HP ($P < 0.05$). The plasma osmotic pressure was significantly lower in the water ingestion trial as compared to that in the non-ingestion trial in both menstrual cycle phases ($P < 0.01$). The serum sodium concentrations during exercise were significantly increased in both phases of the non-ingestion trial, whereas these concentrations were not changed in both phases of the water ingestion trial. The serum sodium concentrations in the water ingestion trial were significantly lower than those in non-ingestion trial in each menstrual cycle phase. The hemoglobin concentration was significantly increased during exercise only during the LP of the

non-ingestion trial. There was no difference between the menstrual cycle phases and between the trials with regard to the hemoglobin concentration, hematocrit, and rate of change in plasma volume before and after exercise.

Table 1-2. Body weight and blood variables before and after exercise

	Non-ingestion trial		Water ingestion trial	
	LP	HP	LP	HP
Body weight (kg)				
before	50.04 ± 4.74	50.25 ± 4.58	50.31 ± 5.04	50.53 ± 4.60
after	49.27 ± 4.69 **	49.33 ± 4.51 **	50.28 ± 5.18 #	50.59 ± 4.62 ##
Plasma osmotic pressure (mosmol/kg)				
before	283.4 ± 3.0	279.6 ± 2.3	281.2 ± 2.8	282.4 ± 1.5
after	287.2 ± 1.6 *	284.8 ± 2.6 *	279.0 ± 2.8 ##	278.2 ± 2.8 *, ##
Serum sodium concentration (mEq/L)				
before	138.8 ± 1.5	138.0 ± 0.7	138.6 ± 1.1	139.2 ± 0.4
after	140.8 ± 1.1 *	140.2 ± 1.9 *	137.8 ± 1.8 #	137.6 ± 1.3 #
Hemoglobin concentration (g/dL)				
before	12.8 ± 1.0	12.9 ± 1.1	13.0 ± 1.1	12.5 ± 0.8
after	13.4 ± 1.1*	13.2 ± 0.9	13.3 ± 0.9	12.6 ± 1.0
Hematocrit (%)				
before	39.0 ± 2.5	40.0 ± 2.6	39.7 ± 2.6	38.4 ± 1.3
after	40.5 ± 3.1	40.4 ± 2.3	40.3 ± 2.5	38.4 ± 2.1
Plasma volume change rate (%)				
after	-1.1 ± 0.8	-1.2 ± 0.7	-0.7 ± 1.3	-1.0 ± 0.6

LP, low progesterone level phase; HP, high progesterone level phase. Date shows average ± standard deviation. *Different compared to that before exercise ($P < 0.05$), **Different compared to that after exercise ($P < 0.01$), #Different compared to that in the non-ingestion trial in same phase ($P < 0.05$), # #Different compared to that in the non-ingestion trial in the same phase ($P < 0.01$)

4.3. Rectal temperature change

Rectal temperature data during exercise are shown in Figure 1-1. There was a significant trial × time interaction ($P < 0.05$), a main effect of trial ($P < 0.01$), and a main effect of time ($P < 0.01$) according to the 4 × 7 (trial × time) two-way repeated ANOVA. Rectal temperature during the HP was significantly higher than that in the LP during exercise in the non-ingestion trial ($P < 0.01$) and in the water ingestion trial ($P < 0.05$). There was no menstrual cycle phase × time interaction in both water conditions. Although a water condition × time interaction effect was noted during the HP ($P < 0.05$), no main effect of water condition or water condition × time interaction was noted during the LP.

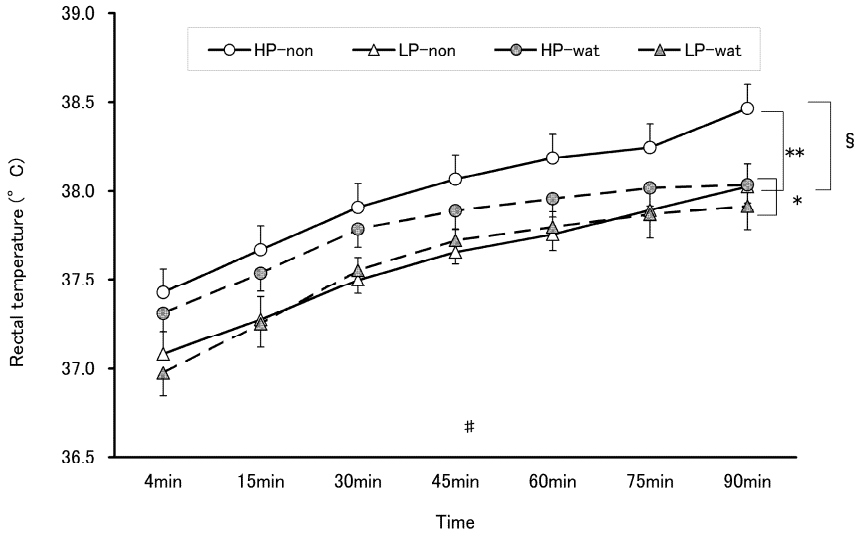

Figure 1-1. Rectal temperature changes during exercise in hot conditions. Data are

presented as mean ± SEM (n = 7). *Significant main effect difference according to repeated-measures ANOVA between menstrual cycle phases in the non-ingestion trial ($P < 0.05$). **Significant main effect difference according to repeated-measures ANOVA between menstrual cycle phases in the water ingestion trial ($P < 0.01$). [§]Significant interaction effect (water condition × time) according to the repeated-measures ANOVA during the HP ($P < 0.05$). [#]Significant main effect of time according to the repeated-measures ANOVA ($P < 0.01$). Abbreviations: HP-non, high progesterone level phase non-ingestion trial; LP-non, low progesterone level phase non-ingestion trial; HP-wat, high progesterone level phase water ingestion trial; LP-wat, low progesterone level phase water ingestion trial.

4.4. Cardiorespiratory responses

The heart rate data during exercise is shown in Figure 1-2. In the 4 × 7 (trial × time) two-way repeated ANOVA, a significant trial × time interaction ($P < 0.05$) and a main effect of time ($P < 0.01$) were noted. There was no significant difference between the menstrual cycle phases in both water conditions. Although a water condition × time interaction effect was noted during the HP ($P < 0.01$), no main effect of water condition or water condition × time interaction was noted during the LP. $\dot{V}$E data during exercise are shown in Figure 1-3. There was no trial × time interaction and no main effect of trial according to the 4 × 7 (trial × time) two-way repeated ANOVA. Furthermore, the RER data during exercise are shown in Figure 1-4. The 4 × 7 (trial × time) two-way repeated ANOVA showed no trial × time interaction and no main effect of trial. PRE data are

provided in Figure 1-5. No differences in overall RPE, cardiovascular RPE, or leg RPE were noted among the four trials at any specific time points.

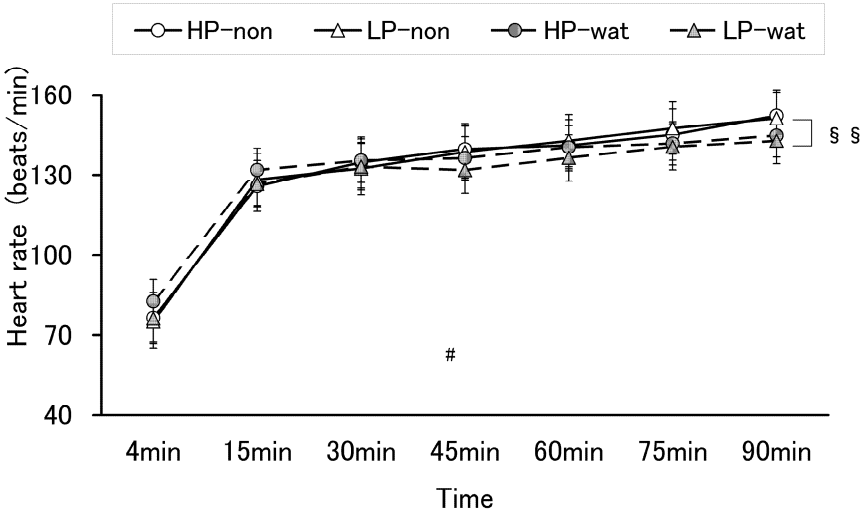

Figure 1-2. Changes in heart rate during exercise in hot conditions. Data are presented as mean ± SEM (n = 7). [§§]Significant interaction effect (water conditions × time) during the HP according to repeated-measures ANOVA ($P < 0.01$). [#]Significant main effect of time according to repeated-measures ANOVA ($P < 0.01$). Abbreviations: HP-non, high progesterone level phase non-ingestion trial; LP-non, low progesterone level phase non-ingestion trial; HP-wat, high progesterone level phase water ingestion trial; LP-wat, low progesterone level phase water ingestion trial.

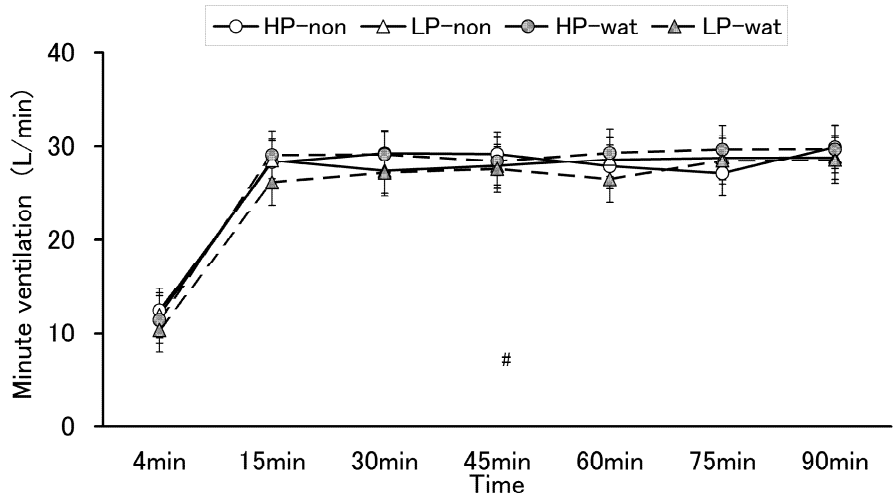

Figure 1-3. Minute ventilation changes during exercise in hot conditions. Data are presented as mean ± SEM (n = 7). Abbreviations: HP-non, high progesterone level phase non-ingestion trial; LP-non, low progesterone level phase non-ingestion trial; HP-wat, high progesterone level phase water ingestion trial; LP-wat, low progesterone level phase water ingestion trial.

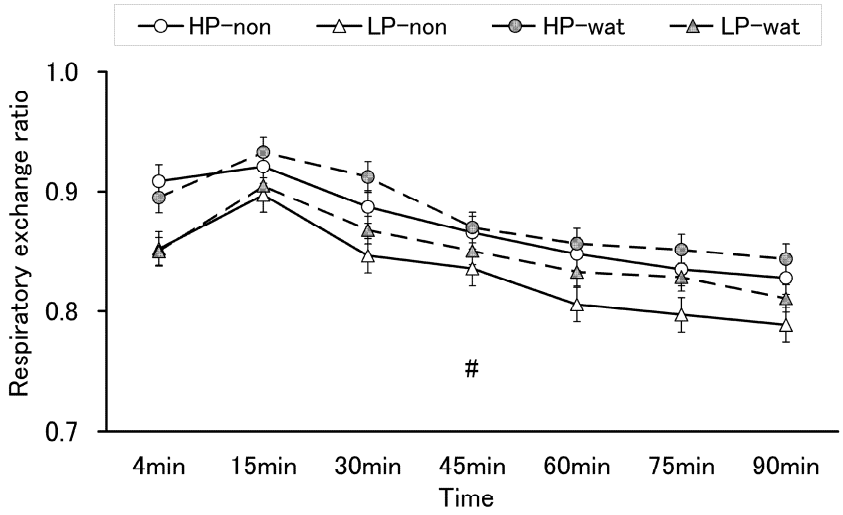

Figure 1-4. Changes in respiratory exchange ratio during exercise in hot conditions. Data are presented as mean ± SEM (n = 7). Abbreviations: HP-non, high progesterone level phase non-ingestion trial; LP-non, low progesterone level phase non-ingestion trial; HP-wat, high progesterone level phase water ingestion trial; LP-wat, low progesterone level phase water ingestion trial.

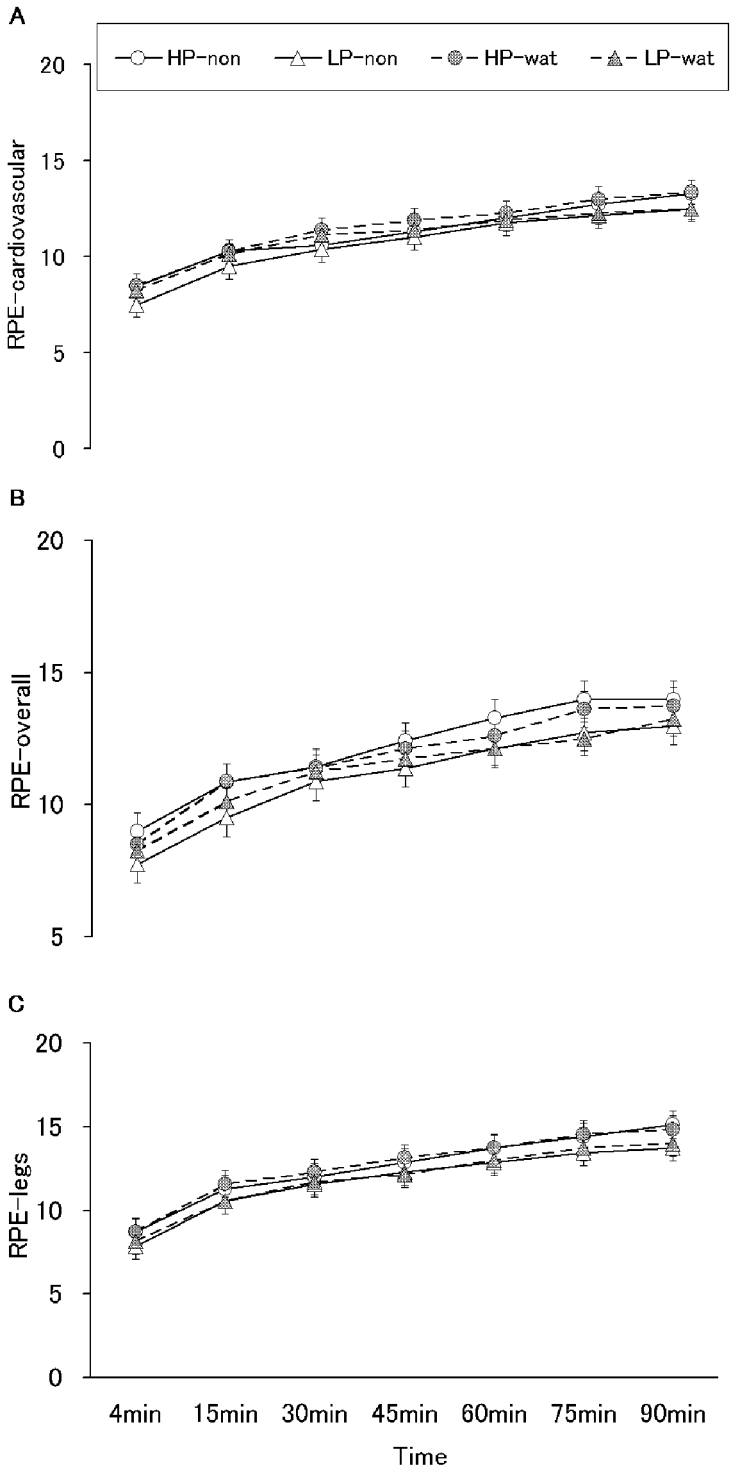

Figure 1-5. Rating of perceived exertion during exercise in hot conditions. Data are presented as mean ± SEM (n = 7). A indicates the RPE-cardiovascular, B indicates the RPE-overall, and C indicates the RPE-legs data. The scale consists of 15 grades, from 6 to 20; 7 = Very, very light, 9 = Very light, 11 = Fairly light, 13 = Somewhat hard, 15 =

Hard, 17 = Very Hard, 19 = Very, very hard. Abbreviations: HP-non, high progesterone level phase non-ingestion trial; LP-non, low progesterone level phase non-ingestion trial; HP-wat, high progesterone level phase water ingestion trial; LP-wat, low progesterone level phase water ingestion trial.

4.5. Correlations

The Pearson correlation coefficients between variables and estradiol levels and progesterone levels were determined. We used data from all subjects (N = 8) in this analysis. A significant negative correlation was found between the estradiol levels and the change in rectal temperature at 75 min (r = −0.631, P = 0.012, n = 15) and 90 min (r = −0.770, P = 0.001, n = 15) only during the LP (all data concerning both water conditions). The relationship between estradiol levels and the degree of increase in rectal temperature at 90 min during the LP is shown in Figure 1-6.

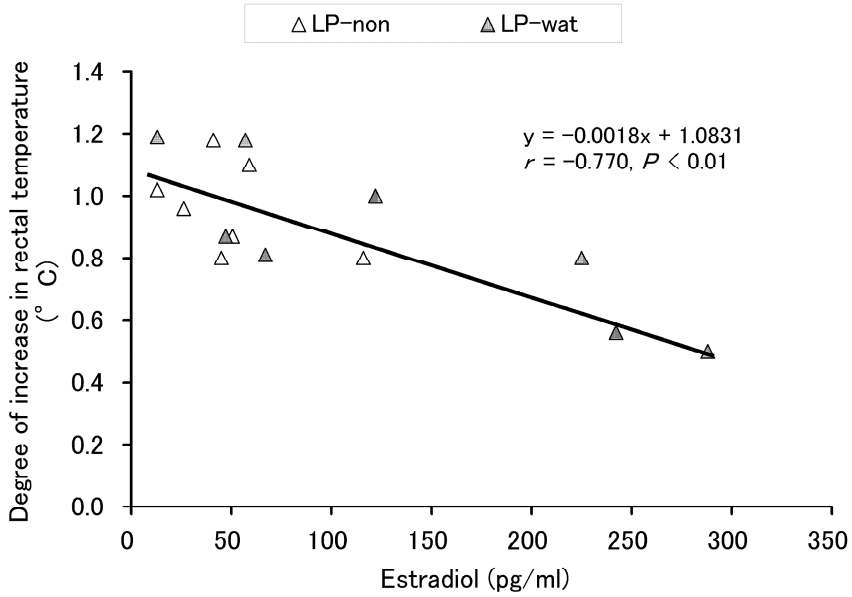

Figure 1-6. Effect of estradiol on rectal temperature in the low progesterone level phase. The relationship between estradiol levels and the degree of increase in rectal temperature at 90 min in the low progesterone level phase. Open triangles represent data from individuals in the non-ingestion trial and black-filled triangles represent data from individuals in the water ingestion trial. Abbreviations: LP-non, low progesterone level phase non-ingestion trial; LP-wat, low progesterone level phase water ingestion trial.

5. Discussion

In the present study, we aimed to investigate the effect of the menstrual cycle phase and water ingestion on physiological responses during prolonged exercise at moderate intensity under hot conditions. We found that rectal temperature during the HP was significantly higher than that in the LP during 90 min of moderate intensity exercise regardless of the presence of water ingestion. Our result suggested that there was no difference in the thermoregulatory responses between the menstrual cycle phases as the rectal temperature increased at the same rate in the both phases regardless of the presence of water ingestion. Most previous studies reported that there was no difference between the menstrual cycle phases in terms of thermosensitivity during exercise [3,17, 18,26], whereas some studies reported that there was a difference between the menstrual cycle phases in terms of thermosensitivity in the absence of water ingestion [16,17]. Pivarnik et al. reported a greater increase in rectal temperature in the luteal phase during

60 min of exercise at 65% $\dot{V}O_2$max at room temperature (22 ºC) without water ingestion [17]. In the previous study, the rectal temperature continued to increase in the luteal phase, whereas rectal temperature reached 38.3°C after 30 min of exercise in the follicular phase. However, the difference between the follicular phase and luteal phase remained constant for 30 min, and the rectal temperature in the luteal phase at 30 min was approximately 38.5°C. In the present study, rectal temperature at 90 min in the non-ingestion trial were 38.5 [SD 0.3] °C during the HP and 38.0 [SD 0.3] °C during the LP. Therefore, our data seem to support the findings of the previous study. Kuwahara et al. reported a weaker thermosensitivity in the luteal phase than in the follicular phase during 30 min of exercise at 50% $\dot{V}O_2$max at 25°C [16]. They suggest the possibility that the lower plasma volume in the luteal phase, compared to that in the follicular phase, led to the lower thermosensitivity in the luteal phase. In the present study, we found that there was no difference between the LP and HP in terms of the hematocrit and hemoglobin at rest, which suggest that there was no difference in the plasma volume between the menstrual cycle phases. They also suggested that high level of heat stress and higher workload would mask the influence of the menstrual cycle phase. Thus, the difference in ambient temperature (30°C vs. 25°C) and exercise

duration (90 min vs. 30 min) may mask the influence of the menstrual cycle phase and lead to a discrepancy in the result. The increase in the rectal temperature was approximately 1.1°C in the present study but was approximately 0.4°C in previous studies, which may be due to this discrepancy.

Garcia et al. reported that the changes in body temperature during exercise under hot and humid conditions may be due to water ingestion, and accordingly dismissed the difference in rectal temperature between the follicular and luteal phase; they reported that these changes may be due to the lower urinary volume and higher sweat volume in the luteal phase compared with the follicular phase [21]. This result may suggest that water reabsorption in the kidney is enhanced in the luteal phase. The current results indicate that the plasma osmotic pressure after exercise during the HP (luteal phase) in the water ingestion trial was significantly decreased as compared to that before exercise, which may suggest the enhancement of water reabsorption in the kidney. Hence, although there was no difference in weight loss during the exercise between different menstrual cycle phases (0.98 [SD, 0.58] kg during the HP and 0.74 [SD, 0.37] kg) in the water ingestion trial, it is possible that the net sweat volume in the luteal phase was greater than that in the follicular phase during exercise and that the heat dissipation was

enhanced in the luteal phase during exercise with water ingestion. Our data revealed that the average difference between the HP and LP was decreased from 0.32°C before exercise to 0.12°C at the end of 90 min of exercise. These data may suggest that the sweat rate was increased in the luteal phase during exercise in the presence of water ingestion.

During the LP, water ingestion did not influence the change in rectal temperature during 90 min of exercise. However, during the HP, water ingestion suppressed the increase in rectal temperature during 90 min of exercise. The unique findings of this study included the discovery of a correlation between blood estradiol levels and the increase in rectal temperature during the LP. Moreover, a negative correlation was observed between estradiol levels and the increase in rectal temperature after 75 min of exercise. The mechanism underlying the effect of estradiol on rectal temperature is believed to involve endothelial cells because the vasodilatory effect of estradiol has been reported previously [27-29]. According to these previous studies, estradiol leads to the production of nitric oxide via the activation of the estradiol receptor on cell membranes in the vessel wall. We consider that the increase in blood circulation caused by estradiol-mediated vasodilation leads to the suppression of the increase in rectal

temperature in an estradiol level-dependent manner. However, we did not observe any effect of estradiol-mediated vasodilatation during the HP, although this may be due to the effect of progesterone. Furthermore, suppression of the increase in rectal temperature led to a suppression of an increase in HR. We found a significant correlation between the increase in rectal temperature and the increase in HR ($r = 0.653$, $P < 0.001$, n = 32), which support the findings of previous studies [30].

6. Conclusion

During 90 min of exercise at moderate intensity under hot conditions, rectal temperature was significantly higher during the HP than during the LP regardless of presence of water ingestion Water ingestion is likely useful for suppressing the increase in body temperature and HR, particularly during the HP, whereas estradiol appears to be useful for suppressing the increase in rectal temperature during the LP.

Chapter2. The effect of the menstrual cycle phase and carbohydrate ingestion on inflammatory response during prolonged exercise under hot conditions

1. Background

With an increase in the number of women participating in sports for recreation, health, fitness, weight management, social interaction, competition, and/or personal accomplishment, the influence of menstrual cycle phase on physiologic response to exercise has received much attention, not only for athletes but also women in general. Sex hormones are known to regulate substrate utilization [31], muscle fatigue [32], temperature regulation [33], and endocrine response [34] during exercise. Evaluation of the differences in exercise response with regard to menstrual cycle phase will help understand the menstrual cycle phase–specific adaptations to exercise and athletic performance.

A number of studies involving male subjects have reported that exercise causes disturbances in circulating leukocyte concentrations and function and that these effects are dependent on the intensity of exercise and the associated release of stress hormones

[35]. Furthermore, body temperature has been shown to affect leukocyte mobilization, cytokines, and markers of neutrophil activation during and after exercise in men. Thus, greater systemic mobilization was observed in a hot environment [36,37].

In women with a normal menstrual cycle, core temperature (Tc) rises by 0.3°–0.5°C in the luteal phase compared to the follicular phase [1]. Therefore, we hypothesized that a stressful condition (higher Tc and cardiorespiratory strain) in the luteal phase would affect immune response to prolonged exercise. Although several studies have reported the effect of exercise on immune response, very few have investigated the effect of menstrual cycle phase. One study reported that menstrual cycle phase did not significantly affect immune cell response (leukocytes, monocytes, neutrophils, and lymphocytes) after 90 min of cycling at 65% maximal aerobic power [38], However, there was no information on Tc during exercise, and it appeared that the concentration of progesterone, which is involved in body temperature regulation, was too low in the luteal phase.

Several studies have investigated the effect of menstrual cycle phase on endurance exercise performance, but consistent results have not been obtained [26,39].

One study showed that endurance performance was significantly decreased during the luteal phase compared with the follicular phase in a hot and humid condition (32°C, 60% relative humidity), even though there was no difference between menstrual cycle phases in a temperate condition (20°C, 45% relative humidity) [20]. One characteristic of that study was to set a strict progesterone concentration (>5.1 ng/mL) as the criterion threshold for definition of the luteal phase.

A number of previous studies involving male subjects reported that CHO ingestion during exercise could suppress the mobilization of leukocytes into the circulation [40-42] and secretion of cytokines [40, 42-45]. Ingestion of CHO during prolonged exercise, maintains blood glucose concentration, lessens hypothalamic-pituitary-adrenal activation and diminishes the perturbation of circulating leukocyte concentration and function [46-48].

It is known that ovarian hormones can exert metabolic actions affecting substrate utilization. For example, administration of estradiol and progesterone in rats has been reported to decrease gluconeogenesis from alanine and to increase hepatic storage [49], and variations in plasma ovarian hormones concentrations have been

shown to alter gluconeogenesis [50]. Furthermore, animal studies show that larger lipid, and lower CHO, utilization occurs during exercise when estrogen and progesterone, are elevated [51,52]. Exercise substrate utilization in women throughout menstrual cycle has generally been characterized by the measurement of respiratory exchange rate (RER). Although discrepancies occur when studying RER in women at rest or during exercise, some data shows the significant difference between the menstrual cycle phases. Furthermore, previous research shows decrease of blood glucose concentration in luteal phase compared to follicular phase during prolonged exercise [53]. This low blood glucose concentration in the luteal phase may have a different impact on immune response between menstrual phases during prolonged exercise.

The first purpose of our study was to examine the effect of menstrual cycle phase on immune response to exercise in a hot condition, with a progesterone concentration threshold for luteal phase verification. To our knowledge, the interaction between exercise in a hot condition and menstrual cycle phase with progesterone limitation on immune response has not been systematically studied because the exercise conditions in previous studies were not defined. The second purpose of our study was to

investigate the effect of CHO ingestion on immune response. We hypothesized that menstrual cycle may affect immune responses and that CHO ingestion attenuates these effects.

2. Material and methods

2.1. Subjects

Six healthy young women volunteered to take part in this study. All subjects maintained a regular menstrual cycle and were not taking any oral contraceptives before testing. Their mean characteristics were as follows: age, 23 (SD, 2.6) years; weight, 48.7 (SD, 6.1) kg; height, 156.2 (SD, 2.4) cm; and peak aerobic power ($\dot{V}O_2$peak), 39.5 (SD, 5.3) mL/kg/min. Four subjects had not performed regular physical activities for the previous 3 years, whereas two other subjects performed regular physical activities (e.g. swimming).

2.2. Experimental design

This study comprised 4 separate experimental trials. Subjects exercised in the condition (30 [SD, 2]°C and 50 [SD, 5]% relative humidity) during follicular and luteal phases of

their menstrual cycle. During the exercise, subjects either consumed a CHO beverage containing 3.8% CHO (2.1% glucose and 1.7% fructose) or a placebo sweetened with an artificial sweetener (sucralose and acesulfame potassium) that tasted like the CHO beverage. We used a hypotonic CHO beverage (osmolality 195 mOsm/kg), which was reported to attenuate some inflammatory responses to exercise [54]. The components and ingredients of both beverages were otherwise identical. The composition of both beverages was as follows: protein and fat, 0 g/100 mL; sodium, 26 mg/100 mL; potassium, 6 mg/100 mL; calcium, 1 mg/100 mL;, and magnesium, 1 mg/100 mL. Both beverages had the same flavor and color (slightly cloudy) and were served to subjects in a transparent plastic cup. Thus, subjects were blinded as to which beverage they were consuming. Subjects were asked to ingest 300 mL of either beverage 30 min before exercise and another 107 mL every 15 min during the 90-min exercise (7 times), so that the total intake was 1,050 mL per participant. The amount and timing of intake were determined according to the position stands of the American College of Sports Medicine [55]. This study was approved by the Human Research Ethics Committee of the Faculty of Sport Sciences of Waseda University for the use of human subjects in

accordance with the Declaration of Helsinki. Prior to participation, each subject provided her informed consent.

2.3. Preliminary testing

To estimate menstrual cycle phase, all subjects recorded their oral temperature upon waking every day for at least 2 months and the day of menstruation for 3 months before commencement of the trial. Additionally, blood samples taken before commencement of the exercise were analyzed for estradiol and progesterone concentrations to determine the menstrual phase. ($\dot{V}O_2$peak) was measured using a maximal graded exercise test with an electromagnetically braked cycle ergometer (Combi RS-232; Combi, Tokyo, Japan). The initial workload was 0 W for 4 min (warming up) and was increased by 30 W every 3 min thereafter, starting at 40 W, until subjects could no longer maintain the required pedaling frequency (70 rpm). Heart rate (HR) was monitored by electrocardiography (Cardiosuper 2E32; Sanei-Sokki, Yamagata, Japan) throughout the exercise. During the progressive exercise test, the expired gas of subjects was collected, and the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production $\dot{V}CO_2$ were measured and averaged over 30-s intervals using an automated breath-by-breath gas analyzer (Minato AE300; Minato Medical Science, Osaka, Japan). $\dot{V}O_2$peak was defined as the highest 30-s value. At the end of each workload stage, subjects were asked to indicate the rating of perceived exertion (RPE) by using the Borg Scale [24].

2.4. Experimental trials

All subjects completed four separate experimental trials, with each trial occurring at a specific time during the menstrual cycle, previously determined for each subject by her basal body temperature. For the 2 CHO and 2 placebo trials, 1 trial each occurred in the follicular phase (FC and FA, respectively), and 1 trial each in the luteal phase (LC and LA, respectively). To avoid a confounding phase and beverage effect with trial order, subjects were randomly assigned trial orders, with 3 subjects commencing in the follicular phase and 3 in the luteal phase. Each experimental trial was performed on a separate day at least 1 week apart. Subjects were asked to only drink water after 21:00 h on the day before the experimental trial, and they ate a standardized breakfast (protein, 12.4 g; fat, 5.5 g; CHO, 75.7 g; and total energy, 395 kcal) at 06:00 h, i.e. 6–7 h before each trial. Thereafter, foods and beverages, except for water, were not allowed.

In all 4 trials, subjects cycled at 50% $\dot{V}O_2$peak (60 [SD, 12.2] W) for 90 min in a hot condition (30 [SD, 2]°C and 50 [SD, 5]% relative humidity) and completed POST. The workload corresponding to 50% $\dot{V}O_2$peak was determined from the graded exercise test by interpolation from the line of the best fit describing the relationship

between power output and $\dot{V}O_2$. During endurance sports competitions, such as marathons, many participants attempt sprints in the final stage of the race. Therefore, in order to simulate an actual competition, our experimental protocol comprised of 2 parts: 90 min of cycling exercise at moderate intensity and a timed performance test. This study composed of a prolonged exercise and performance test under hot condition by untrained subjects. We set exercise intensity at 50% $\dot{V}O_2$peak, which was lower than the 65% $\dot{V}O_2$max and room temperature condition set in the previous study [9] because we thought that the 65% $\dot{V}O_2$max intensity under hot condition would be too strenuous for untrained subjects. To measure rectal temperature during the exercise, subjects self-inserted a rectal probe (401 J; Nikkeiso-YSI Co. Ltd., Musashino, Japan) 10 cm past the anal sphincter. During exercise, minute ventilation ($\dot{V}E$), expired gas concentration, HR, and rectal temperature were measured for 3 min at the 4-min (warm-up), 15-min, 30-min, 45-min, 60-min, 75-min, and 90-min time points. Subjects were asked to indicate their overall RPE, RPE-cardiovascular, and RPE-legs to identify specific locations of perceived exertion at every 15-min time point from the warm-up to the end of the 90-min cycling exercise.

Following the 90-min exercise, subjects completed POST that lasted approximately 10 min in the same condition. Subjects were required to complete a set amount of work (52.4 [SD, 8.6] kJ) as fast as possible. The total amount of work to be performed was calculated using the following formula [56]: Total work (J) = 0.65 Wpeak × 600. Wpeak (134.4 [SD, 22] W) was the maximal workload capacity determined in preliminary testing and 600 was the duration in seconds (equivalent to 10 min). The ergometer was connected to a computer that calculated and displayed the total amount of work performed. Subjects received only information on the percentage of work performed relative to the set amount of work from the examiner. A familiarization trial was also completed before commencement to allow subjects to familiarize themselves with the protocol and laboratory setting.

2.5. Blood sampling and analysis

Venous blood samples were collected by venipuncture from an antecubital vein before exercise (PRE); at the 30-min, 60-min, and 90-min time points during the exercise; and at POST. Blood samples were collected into serum separation tubes or vacutainers containing ethylenediaminetetraacetic acid (EDTA). A fraction of whole blood was

used to measure hemoglobin, hematocrit, and full blood cell count. Serum separation tubes were left to allow blood to clot at room temperature for 30 min, while vacutainers containing EDTA for plasma separation were immediately centrifuged at 1,000 × *g* for 10 min. Serum and plasma were then removed and stored at -80°C for future analysis. Serum free fatty acid and plasma glucose concentrations, leukocyte concentrations, hemoglobin, and hematocrit were analyzed by BML, Inc. (Tokyo, Japan). Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure plasma concentrations of the cytokines interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), IL-6, tumor necrosis factor (TNF)-α (R&D Systems, Minneapolis, MN), IL-8, IL-10, and IL-12p40 (Becton Dickinson Bioscience, San Diego, CA), and the neutrophil activation markers myeloperoxidase (MPO) and calprotectin (HyCult Biotechnology, Uden, the Netherlands). ELISA measurements were performed according to the instructions for each ELISA kit using a microplate reader (VERSAmax; Molecular Devices, Sunnyvale, CA). Plasma concentrations of all these variables were adjusted for changes in plasma volume [57].

2.6. Statistical analysis

All data were checked for normal distribution using the Kolmogorov-Smirnov statistic. Data for rectal temperature, HR, VE, RER, blood glucose, serum free fatty acid, leukocyte concentrations, and performance test result were normally distributed. Data for serum sex hormones, IL-8, IL-12p40, and MPO concentrations were normally distributed after log transformation. Data for serum IL-1β, IL-1ra, IL-6, IL-10, calprotectin, and TNF-α concentrations were not normally distributed. Normally distributed data (sex hormones and POST result were analyzed using a 2 × 2 factor (menstrual cycle phase × beverage) repeated analysis of variance (ANOVA). For other normally distributed data (rectal temperature, HR, and RER), a 4 × 8 factor (trial × time) repeated ANOVA was used to determine trial effects, time effects, and trial × time interactions. For other normally distributed data (blood glucose, free fatty acid, leukocyte concentrations, IL-8, IL-12p40, and MPO), a 4 × 5 factor (trial × time) repeated ANOVA was used to determine trial effects, time effects, and trial × time interactions. When significant trial effects, time effects, or trial × time interactions were evident, Bonferroni posthoc multiple comparisons were used. Data for serum IL-1β, IL-1ra, IL-6, IL-10, calprotectin, and TNF-α were analyzed using nonparametric Friedman's ANOVA on ranks test to determine time effects. Kruskal-Wallis' one-way

ANOVA was used to assess differences between trials at specific time points. Data were analyzed using SPSS version 19 for Windows (IBM Corporation, Armonk, NY) with the threshold for statistical significance set at $P = 0.05$. Relationships between dependent variables and leukocyte concentration were assessed with Pearson product correlations. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Hormones

Table 2-1 shows the serum estradiol and progesterone concentrations of all subjects. Menstrual cycle phase × beverage did not affect serum estradiol and progesterone concentrations. A main effect of menstrual cycle phase ($P < 0.01$), but not of the beverage, on both hormones was observed. Serum estradiol and progesterone concentrations were significantly higher in the luteal phase than in the follicular phase [58]. We used the progesterone concentration limit 5.1 ng/mL for luteal phase verification according to previous studies [20,23].

Table 2-1. Resting hormone concentration of subjects before each trial

Subject	FA		LA		FC		LC	
	[E] (pg/mL)	[P] (ng/mL)	[E] (pg/mL)	[P] (ng/mL)	[E] (pg/mL)	[P] (ng/mL)	[E] (pg/mL)	[P] (ng/mL)
1	33.4	0.2	171.4	18.9	163.7	0.3	321.3	15.7
2	34.8	0.2	125.5	10.4	42.7	0.1	98.5	7.4
3	30.8	0.4	94.0	6.0	39.4	0.2	68.3	5.7
4	47.3	0.1	108.9	6.4	83.8	1.4	171.0	14.9
5	50.6	0.2	201.4	13.2	98.0	0.2	327.4	19.9
6	201.0	0.4	151.2	25.6	44.0	0.4	102.1	9.1
mean	66.3	0.3	142.1	13.4	78.6	0.4	181.4	12.1
SD	66.5	0.1	40.4	7.0	48.3	0.4	115.7	5.1

Abbreviations: FA, follicular phase placebo trial; LA, luteal phase placebo trial; FC, follicular phase carbohydrate trial; LC, luteal phase carbohydrate trial. [E], estradiol concentration; [P], progesterone concentration.

3.2. Physiologic response

Rectal temperature data during exercise is shown in Figure 2-1. The results show that menstrual cycle phase and beverage type had significant effect on rectal temperature during exercise ($P<0.05$). Although there was a significant difference within a trial,

there was no significant difference between the trials. Rectal temperature was

significantly increased over time during exercise ($P < 0.01$).

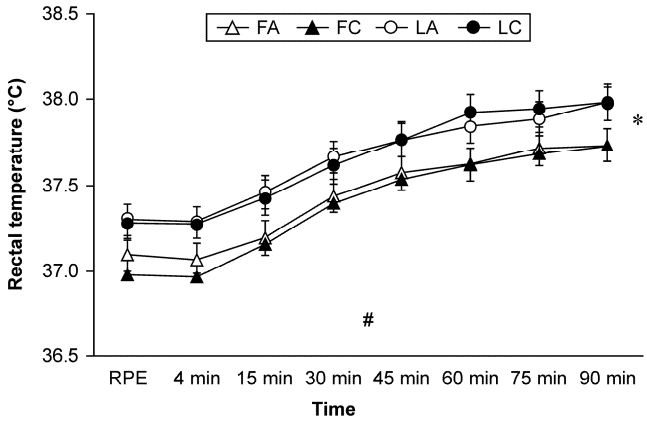

Figure 2-1. Rectal temperature during cycling exercise

Data are mean ± SEM (n = 6). *Significant repeated-measures ANOVA main effect difference between the trials ($P < 0.05$). #Significant repeated-measures ANOVA main effect in time ($P < 0.01$). Abbreviations: FA, follicular phase placebo trial; FC, follicular phase carbohydrate trial; LA, luteal phase placebo trial; LC, luteal phase carbohydrate trial; PRE, before exercise.

Table 2-2 shows cardiorespiratory measures. The results show that menstrual

cycle and beverage type had no significant effect on HR, $\dot{V}$□E, and RER. However HR

and $\dot{V}$□E significantly increased to over time during exercise ($P < 0.01$). RER

significantly changed over time during exercise ($P < 0.01$). Furthermore, there was no

significant effect of menstrual cycle phase or beverage type on RPE data (data not

shown).The total amount of CHO consumed in the CHO trials (FC and LC) was 40 g.

Plasma glucose and free fatty acid data are shown in Figure 2-2. There was no

trial × time interaction with respect to change in glucose; however, there was a main effect in trial ($P < 0.05$) and time ($P < 0.01$). Blood glucose at 90 min in the LA was significantly lower than that in the LC ($P < 0.05$), and a lower trend compared with that in the FC ($P < 0.068$).

There was a trial × time interaction ($P < 0.05$), a main effect in time ($P < 0.01$), and a trend in trial ($P = 0.082$) in serum free fatty acid. The concentration at POST in the LA was significantly higher compared with that in the FC ($P < 0.01$).

Table 2-2. Cardiorespiratory measures during 90 min exercise

	Trial	4 min	15 min	30 min	45 min	60 min	75 min	90 min
HR	FA	80 ± 50	131 ± 11	138 ± 12	140 ± 12	145 ± 12	149 ± 15	154 ± 15
	FC	83 ± 12	134 ± 14	143 ± 17	145 ± 20	148 ± 19	153 ± 21	156 ± 21
(beat/min)	LA	82 ± 40	137 ± 90	142 ± 11	149 ± 13	148 ± 18	154 ± 13	156 ± 17
	LC	91 ± 16	141 ± 12	149 ± 12	150 ± 12	153 ± 15	154 ± 14	156 ± 14
$\dot{V}$**E**	FA	11.1 ± 1.3	28.4 ± 2.4	29.6 ± 3.4	28.4 ± 4.5	29.8 ± 3.8	29.5 ± 3.3	29.8 ± 3.0
	FC	10.9 ± 1.0	28.7 ± 2.6	29.5 ± 2.3	28.9 ± 3.1	28.6 ± 2.3	30.0 ± 3.3	30.6 ± 3.5
(L/min)	LA	10.9 ± 2.6	28.9 ± 1.9	30.1 ± 2.4	30.2 ± 4.0	31.0 ± 4.3	32.5 ± 4.9	33.2 ± 6.1
	LC	12.1 ± 1.0	29.1 ± 5.4	30.4 ± 3.4	30.5 ± 4.3	30.4 ± 4.5	30.7 ± 4.5	31.9 ± 4.8
RER	FA	0.81 ± 0.04	0.88 ± 0.03	0.88 ± 0.03	0.83 ± 0.05	0.84 ± 0.04	0.82 ± 0.04	0.80 ± 0.04
	FC	0.83 ± 0.08	0.89 ± 0.04	0.87 ± 0.04	0.83 ± 0.04	0.82 ± 0.04	0.82 ± 0.04	0.81 ± 0.04
	LA	0.84 ± 0.05	0.90 ± 0.07	0.89 ± 0.06	0.86 ± 0.08	0.85 ± 0.06	0.85 ± 0.07	0.84 ± 0.08
	LC	0.85 ± 0.11	0.89 ± 0.06	0.88 ± 0.05	0.86 ± 0.04	0.85 ± 0.05	0.84 ± 0.05	0.84 ± 0.05

Dates are shown as mean ± SD. Abbreviations: FA, follicular phase placebo trial; FC, follicular phase carbohydrate trial; IL, interleukin; LA, luteal phase placebo trial; LC,

luteal phase carbohydrate trial; HR, heart rate; $\dot{V}$E, minute ventilation; RER, respiratory exchange rate.

3.3. Inflammatory response

Leukocyte concentration data are shown in Figure 2-3. There was a trial × time interaction ($P<0.01$), and a main effect in trial ($P<0.01$) and time in leukocyte concentration ($P<0.01$). Leukocyte concentration at 90 min in the LA was significantly higher than that in the FC ($P<0.01$), and that at POST in the LA were significantly higher than those in the FA ($P<0.05$), LC ($P<0.01$), and FC ($P<0.01$). Leukocyte concentrations at 90 min in the LA was higher than those in the FA ($P=0.073$) and LC ($P=0.098$). Moreover, leukocyte concentrations at POST in the FA were significantly higher than those in the FC ($P<0.01$). Pearson's correlation coefficient between serum free fatty acid concentration and leukocyte concentration was determined, and a significant positive correlation was found ($r=0.661$, $P<0.001$).Serum cytokine concentration data are shown in Table 2-3. Serum IL-6 concentrations were significantly higher at 90 min ($P<0.05$) and POST ($P<0.01$) from PRE in 3 trials (FA, LA, and LC). Moreover, the concentrations at POST in these 3 trials were significantly higher ($P<0.01$) than those at 30 min. In the FC trial, the concentration at POST was

significantly higher compared with that at PRE and 30 min. However, there were no significant differences between the trials at any time point. Serum MPO concentrations were highest at POST in all trials. There was a main effect in time ($P<0.01$), but no trial × time interaction or main effect in trial. Serum calprotectin concentrations were significantly higher ($P<0.01$) at POST from PRE in all 4 trials. The concentrations at POST in the FC ($P<0.05$), LA ($P<0.01$), and LC ($P<0.05$) were higher than those at 30 min. Moreover, the concentrations at 90 min in the FC ($P<0.01$), LA ($P<0.01$), and LC ($P<0.05$) were significantly higher than those at PRE.

Serum IL-1β, IL-1ra, IL-8, IL-10, IL-12p40, and TNF-α concentrations remained unchanged following the exercise in all 4 trials, and no significant differences were observed between trials.

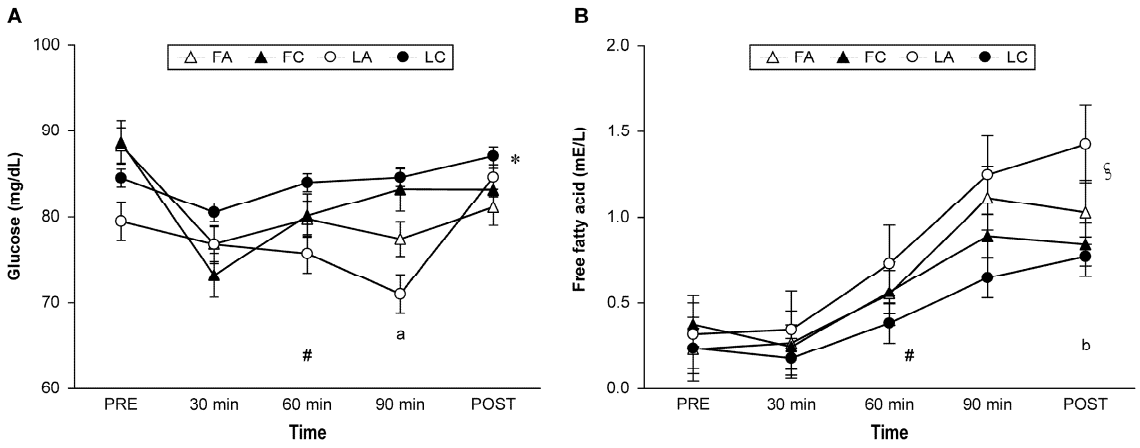

Figure 2-2. Blood glucose and serum free fatty acid concentrations.
Data are mean ± SEM. *Significant repeated-measures ANOVA main effect difference between the trials ($P < 0.05$). [#]Significant repeated-measures ANOVA main effect in time ($P < 0.01$). [a]Significantly different between LA and LC ($P < 0.05$) [§]Significant repeated-measures ANOVA interaction between trial and time ($P < 0.05$). [b]Significantly different between LA and FC ($P < 0.01$). Abbreviations: FA, follicular phase placebo trial; FC, follicular phase carbohydrate trial; LA, luteal phase placebo trial; LC, luteal phase carbohydrate trial; PRE, before exercise; POST, high intensity time trial performance test.

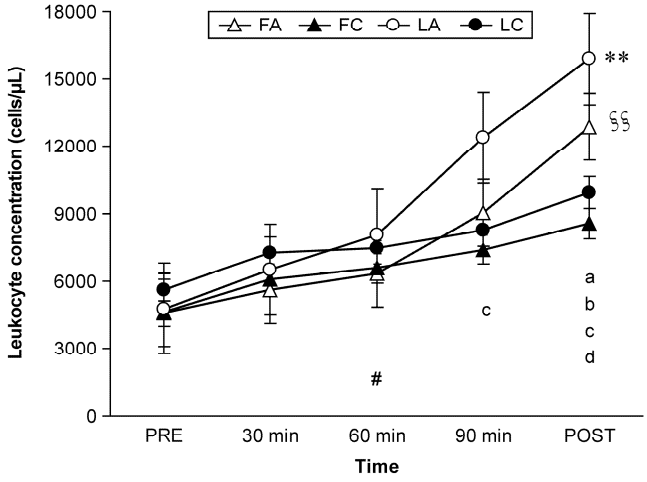

Figure 2-3. Leukocyte concentration during exercise and after performance test.
Data are mean ± SEM. **Significant repeated-measures ANOVA a trial × time interaction ($P < 0.01$). [§§]Significant repeated-measures ANOVA main effect difference between the trials ($P < 0.01$). [#]Significant repeated-measures ANOVA main effect difference in time ($P < 0.01$).[a]Significantly different between LA and FA ($P < 0.05$). [b]Significantly different between LA and LC ($P < 0.01$). [c]Significantly different between LA and FC ($P < 0.01$). [d]Significantly different between FA and FC ($P < 0.01$). Abbreviations: FA, follicular phase placebo trial; FC, follicular phase carbohydrate trial; LA, luteal phase placebo trial; LC, luteal phase carbohydrate trial; PRE, before exercise; POST, high intensity time trial performance test.

Table 2-3. Serum cytokine concentrations

	PRE	30 min	60 min	90 min	POST
IL-1β (pg/mL)					
FA	0.9 ± 3.0	0.9 ± 2.5	1.0 ± 3.2	0.9 ± 2.8	0.7 ± 2.9
FC	0.9 ± 3.9	1.0 ± 4.9	1.0 ± 3.3	1.2 ± 3.7	1.4 ± 3.3
LA	0.9 ± 3.1	1.0 ± 2.8	0.8 ± 2.8	1.1 ± 3.0	1.1 ± 2.8
LC	1.2 ± 2.5	1.1 ± 2.7	0.9 ± 2.4	1.1 ± 2.7	1.3 ± 2.2
IL-1ra (pg/mL)					
FA	186 ± 109	196 ± 131	203 ± 154	256 ± 77	267 ± 130
FC	159 ± 59	173 ± 51	234 ± 95	214 ± 121	212 ± 89
LA	203 ± 103	200 ± 100	202 ± 83	196 ± 61	269 ± 152
LC	234 ± 117	219 ± 138	209 ± 81	224 ± 125	177 ± 115
IL-6 (pg/mL)					
FA	0.2 ± 1.5	0.1 ± 1.3	0.6 ± 2.3	2.6 ± 3.9[a]	6.3 ± 12.9[b,d]
FC	0.1 ± 0.5	0.2 ± 0.3	0.8 ± 1.5	1.9 ± 3.6	2.9 ± 8.8[a,c]
LA	0.2 ± 0.2	0.2 ± 0.1	0.5 ± 0.8	1.5 ± 4.0[a]	2.2 ± 9.2[b,d]
LC	0.2 ± 0.4	0.2 ± 0.2	0.9 ± 1.2	2.3 ± 2.0[a,c]	4.0 ± 6.6[b,d]
IL-8 (pg/mL)					
FA	1.2 ± 0.5	1.5 ± 0.5	1.3 ± 0.7	1.7 ± 0.7	2.0 ± 0.9
FC	1.5 ± 0.5	1.3 ± 0.5	1.0 ± 0.4	1.5 ± 0.5	1.4 ± 0.6
LA	0.8 ± 0.4	1.1 ± 0.4	1.2 ± 0.7	1.2 ± 0.4	1.1 ± 0.5
LC	1.1 ± 0.4	1.5 ± 0.6	1.1 ± 0.5	1.6 ± 0.9	1.8 ± 0.7
IL-10 (pg/mL)					
FA	5.3 ± 4.1	3.8 ± 3.8	5.1 ± 3.8	4.2 ± 1.6	2.8 ± 2.0
FC	2.6 ± 2.3	3.0 ± 5.0	3.2 ± 1.3	2.5 ± 2.7	2.9 ± 3.2
LA	4.5 ± 8.5	3.3 ± 2.4	3.5 ± 3.2	3.5 ± 3.0	4.9 ± 3.4
LC	3.4 ± 3.5	3.7 ± 3.5	3.9 ± 5.6	3.7 ± 2.4	3.4 ± 2.5
IL-12p40 (pg/mL)					
FA	37 ± 8	44 ± 11	36 ± 6	38 ± 9	43 ± 5
FC	29 ± 5	36 ± 4	43 ± 8	27 ± 6	36 ± 6
LA	43 ± 7	37 ± 6	32 ± 4	30 ± 10	35 ± 5

LC	32 ± 8	32 ± 3	33 ± 3	30 ± 3	34 ± 4
Calprotectin (ng/mL)					
FA	21 ± 14	26 ± 19	27 ± 24	47 ± 28	95 ± 147[b]
FC	18 ± 46	21 ± 121	25 ± 137	39 ± 196[b]	43 ± 267[b,c]
LA	23 ± 32	42 ± 29	62 ± 67	170 ± 181[b]	240 ± 117[b,d]
LC	26 ± 17	40 ± 45	60 ± 53	64 ± 42[a]	103 ± 276[b,c]
MPO (ng/mL)					
FA	17 ± 2	20 ± 3	20 ± 1	27 ± 4	46 ± 11 [e]
FC	20 ± 5	26 ± 8	25 ± 5	38 ± 13	39 ± 9
LA	10 ± 2	26 ± 4	24 ± 2	34 ± 3	46 ± 5
LC	17 ± 2	21 ± 3	19 ± 3	30 ± 7	34 ± 8
TNF-α (pg/mL)					
FA	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
FC	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
LA	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
LC	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1

Data for IL-8, IL-12p40 and MPO are shown as mean ± SEM; data for IL-1β, IL-1ra, IL-6, IL-10, Calprotectin and TNF-α are shown as median ± quartile deviation [a]Significantly different from PRE ($P < 0.05$). [b]Significantly different from PRE ($P < 0.01$). [c]Significantly different from 30 min ($P < 0.05$). [d]Significantly different from 30 min ($P < 0.01$). [e]Main effect in time ($P < 0.01$).

Abbreviations: FA, follicular phase placebo trial; FC, follicular phase carbohydrate trial; IL, interleukin; LA, luteal phase placebo trial; LC, luteal phase carbohydrate trial; MPO, myeloperoxidase; POST, high intensity time trial performance test; PRE, before exercise; TNF, tumor necrosis factor.

3.4. Performance test

The mean time (s) required to complete POST was 675 (SD, 163) in the FA, 681 (SD,

147) in the LA, 692 (SD, 156) in the FC, and 678 (SD, 180) in the LC, respectively. There were no significant differences between trials. Subjects indicated an RPE of 20 for all trials at POST.

4. Discussion

The first purpose of the present study was to investigate the effect of menstrual cycle phase on immune response to exercise. The results showed that menstrual cycle phase affected leukocyte concentrations in response to prolonged exercise; leukocyte mobilization in the luteal phase was larger compared to that in the follicular phase at 90 min of cycling and at POST while ingesting a placebo beverage. However, there was no significant effect of menstrual cycle phase on serum cytokine concentrations. Thus, these results partially supported our hypothesis.

The second purpose of this study was to investigate whether CHO ingestion has an effect on immune response to prolonged exercise. The results showed that ingesting a CHO beverage attenuated leukocyte mobilization and eliminated the differences between menstrual cycle phases observed in the placebo beverage trials. However, there

was no effect of CHO ingestion on serum cytokine concentrations, which partially supported our hypothesis.

A previous study showed that there was no significant difference in total leukocyte concentration between menstrual cycle phases after 90 min of cycling exercise [38]. The reason for the conflicting results between the previous and present study may be difference in the range of progesterone concentrations (10.3 [SD, 8.3] nmol/L) in the luteal phase. This range indicates that most subjects had a lower progesterone concentration than the 16 nmol/L limit, which may have weakened the effect of progesterone on body temperature [20]. However, the correlation between Tc and immune response could not be determined because no information on Tc was available in the previous study.

It is well known that there is a substantial increase in leukocyte concentration (mainly neutrophils) during endurance exercise, and this increase depends on the intensity and duration of exercise [59]. It has been shown that the elevation of neutrophils is due to several hormones (e.g., epinephrine, cortisol, growth hormone, and prolactin) that are known to have immunomodulatory effects [60]. We hypothesized

that higher Tc and potentially increased cardiorespiratory strain in the luteal phase would result in a disturbance in immune response. However, the results of this study showed that there were no significant differences in cardiorespiratory responses between menstrual cycle phases even though thermoregulatory response was significantly different between menstrual cycle phases. Therefore, we thought that causes other than Tc and cardiorespiratory strain could increase leukocyte concentrations in the luteal phase.

Some studies have found that menstrual cycle phase does indeed affect hormonal and metabolic responses to exercise [61,62], particularly in a CHO-depleted nutrition state [53,63]. It is possible that high concentrations of sex hormones in the luteal phase decrease gluconeogenesis and blood glucose concentrations, which, in turn, may lead to an increase in circulating cortisol concentrations and leukocytosis. In this study, the blood glucose concentration at 90 min in the LA trial was lower compared with that in the other trials. Moreover, the high correlation between serum free fatty acid concentration and leukocyte concentration may suggest this mechanism. It is possible that the CHO beverage maintained blood glucose concentrations and prevented

the mobilization of stress hormones that cause leukocytosis. Thus, the increase in leukocyte concentration in the LA might be due to the combined effect of exercise and differential substrate metabolism by sex hormones.

Previous studies on male subjects have consistently reported that ingesting CHO during exercise suppresses the rise in circulating neutrophils, most likely by reducing the secretion of stress hormones that regulate neutrophil mobilization by maintaining high blood glucose [40-42]. The results of this study may support these previous studies.

Muscle-derived IL-6 appears to be at least partly responsible for the elevated secretion of cortisol during endurance exercise. Infusion of recombinant human IL-6 into resting humans to mimic the exercise-induced plasma concentrations of IL-6 has been shown to increase plasma cortisol in a similar manner [60,64]. In this study, an increase in IL-6 concentration was observed in all trials, but there were no significant differences between menstrual cycle phases or CHO ingestion at any time point. These results suggest that factors other than IL-6 may be more related to changes in leukocyte concentrations, consistent with a previous study [38].

Most [40,42,44,48,65], but not all, studies [45,66,67] report that carbohydrate ingestion attenuates plasma IL-6 concentration following exercise. The increase in plasma IL-6 concentration observed following all trials in our study could be due to release of IL-6 from the skeletal muscle [68,69]. However, consistent plasma IL-6 concentration between trials was most likely due to consistent release of IL-6 from the skeletal muscle during exercise and suggests that there was no difference in muscle glycogen concentration [70].

Elevated serum MPO concentration after exercise likely reflects neutrophil degranulation because MPO is contained in azurophilic granules within neutrophils. MPO is an important contributor to neutrophil microbicidal activity. Serum MPO concentration depends on exercise intensity [71] and temperature [36]. In the present study, serum MPO concentration was significantly increased at POST compared with PRE, but there were no significant differences between trials. In this study, consistent with other findings [72], CHO intake did not influence change in serum MPO concentration.

Calprotectin is secreted from monocytes and neutrophils in response to a variety of inflammatory conditions [73]. In the present study, serum calprotectin concentration was significantly increased at POST compared with PRE, but there were no significant differences between trials. These results are in agreement with a previous study, in which the effect of heat stress during exercise on change in serum calprotectin concentration was reported [36]. The mechanisms regulating calprotectin release and the biological role of calprotectin during exercise are currently uncertain. We incorporated the POST in the experimental protocol assuming that participants would attempt a sprint similar to that in actual competitions. The study results show that menstrual cycle phase and beverage type had no significant effect on the timed performance test. In this study, cardiorespiratory responses were not significantly different between trials; therefore, no differences in performance between trials could be observed. This result supports a previous study [20], in which menstrual cycle phase did not affect the endurance performance at moderate temperatures.

One of the limitations of this study is the absence of cortisol, growth hormone, catecholamine, and muscle glycogen measurements during exercise, which would have

allowed a better understanding of the relationships among hormone concentrations, glucose availability, and differential leukocyte concentrations. Even though we found that menstrual cycle phase significantly affected leukocyte concentration, we cannot rule out the possibility that the small cohort of subjects used in this study might have had a negative impact on some of the other measurements, rendering them non-significant. The small number of subjects may not have provided adequate statistical power to detect real, but relatively small, differences in some of the measured variables.

5. Conclusion

Menstrual cycle phase affected circulating leukocyte concentrations during endurance exercise while ingesting a placebo. The degree of leukocyte mobilization was greater in the luteal phase compared with the follicular phase. Ingestion of the CHO beverage eliminated the effect of menstrual cycle phase on leukocyte concentration, which may be explained by the effect of sex hormones on substrate utilization. However, there was no effect of menstrual cycle phase on cytokines during exercise.

The results of this study demonstrate that menstrual cycle changes do alter leukocyte concentration during exercise when subjects consume CHO beverage. Indeed, as the placebo trials progressed, differences became more pronounced, indicating that as endogenous CHO reserves were depleted the effect of menstrual cycle changes become more evident. This was further supported by the absences of difference in immune response between the FC and LC trials.

In women undergoing endurance training for recreational purposes, prolonged exercise in the luteal phase may lead to larger immune disturbances compared to the follicular phase if they ingest water alone during the duration of the exercise. Therefore, we recommend ingesting carbohydrate beverages to attenuate immune disturbances especially in the luteal phase, even though they are unlikely to enhance test performance. However, the total subject number is small and does not constitute a representative sample, and the results need further validation using larger cohorts.

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Concluding remarks

This study revealed that water ingestion may be useful to suppress the increase in body temperature, especially during the luteal phase (high progesterone level phase) during prolonged exercise under hot. Furthermore, this study suggested that the disturbance of inflammatory response observed during the luteal phase during prolonged exercise under hot conditions is suppressed by carbohydrate ingestion.

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