

Research article

The Effect of Co-Ingestion of Carbohydrate with Milk after Exercise in Healthy Women: Study Considering the Menstrual Cycle

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Abstract

This study aimed to assess the effects of co-ingestion of carbohydrate with milk (MILK) and isocaloric carbohydrate beverage (CHO) on post-exercise recovery and subsequent exercise capacity, considering the menstrual cycle. This study included 12 women with regular menstrual cycles who completed four test days, which started with glycogen-depleting exercise using a cycle ergometer in the early follicular phase (EF) and late follicular phase (LF), followed by 240 min of recovery from the ingestion of 200 mL of CHO or MILK every 30 min immediately after the exercise (POST0) until 210 min post-exercise. After 240 min, participants performed an exercise capacity test. Blood samples and breathing gas samples were collected before the exercise (PRE), POST0, and 120 (POST120) and 240 min after the end of exercise (POST240) to determine the concentrations of estradiol, progesterone, blood glucose, blood lactate, free fatty acid (FFA), and insulin and the respiratory exchange ratio, fat oxidation, and carbohydrate oxidation. The exercise time at exercise capacity test was not significantly different in terms of menstrual cycle phases and recovery beverages ingested. However, there was a significant positive correlation between the exercise capacity test and area under the curve (AUC) of FFA concentrations from POST0 to POST240 in each group (EF + CHO, $p < 0.05$; LF + CHO, $p < 0.05$; EF + MILK, $p < 0.01$; and LF + MILK, $p < 0.05$). The AUC of FFA from POST120 to POST240 showed no difference between EF (CHO and MILK) and LF (CHO and MILK). However, the AUC of FFA concentrations from POST120 to POST240 was significantly greater in MILK (EF and LF) than that in CHO (EF and LF) ($p < 0.05$). In active women, circulating substrates and hormone concentrations during short recovery post-exercise are not affected by the menstrual cycle. However, MILK may affect circulating substrates during recovery and the exercise capacity after recovery.

Key words: Cycling, free fatty acid, late follicular phase, menstrual cycle, milk, recovery.

Introduction

Prolonged moderate-intensity exercise or high-intensity exercise causes significant muscle glycogen depletion, which leads to diminished performance (Bergström et al., 1967; Tsintzas et al., 1996; Alghannam et al., 2016b). Moreover, athletes must often perform multiple sessions or practice and strenuous activities with a short recovery time. Therefore, it is important to restore muscle glycogen quickly during short-term recovery.

The resynthesis of glycogen post-exercise is largely

influenced by the type, amount, and timing of nutrient intake (Betts and Williams, 2010; Alghannam et al., 2018). Regarding intake timing, it is important to begin feeding immediately after exercise (Ivy et al., 1988). Additionally, carbohydrate supplementation should be continued throughout recovery with more rapid rates of muscle glycogen resynthesis typically achieved when carbohydrates are provided at relatively frequent intervals (every 15 - 30 min) (Doyle et al., 1993; Van Loon et al., 2000; Jentjens et al., 2001). The amount of carbohydrate intake is suggested, and ingesting approximately 1.2 g of carbohydrate·kg BM⁻¹·h⁻¹ is likely to maximize muscle glycogen resynthesis, and additional carbohydrates will not further increase this glycogenic response (Alghannam et al., 2018). The addition of protein or fat to carbohydrates positively affects glycogen resynthesis and subsequent same-day endurance performance (Ivy et al., 2002; Williams et al., 2003; Betts et al., 2007; Alghannam et al., 2016a). A previous study reported a significantly greater muscle glycogen concentration after 4-h of recovery when carbohydrate-protein supplementation was consumed immediately and 2-h post-exercise after the glycogen depletion exercise when compared with carbohydrate supplementation (Ivy et al., 2002). Ingestion of chocolate milk, which was often used as a carbohydrate-protein beverage, results in a longer exercise time after ingestion than after a carbohydrate replacement beverage ingestion or fluid replacement beverage when the participants performed a glycogen-depleting trial, a 4-h recovery period, and a cycle to exhaustion at 70% power at maximal oxygen uptake (Thomas et al., 2009). Recently, the effect of post-exercise milk-based beverage ingestion (milk and co-ingestion of carbohydrate with milk) has been investigated in some studies and has the same effect as that of post-exercise ingestion of carbohydrate-protein beverages and chocolate milk (Sudsa-Ard et al., 2014; Russo et al., 2019; James et al., 2019). Therefore, the use of milk that is cheaper and easier to help many athletes to recover quickly after exercise. However, little information exists on the effects of post-exercise milk consumption on substrate responses and subsequent performance in females (Sudsa-Ard et al., 2014; Russo et al., 2019).

Women have a menstrual cycle that is characterized by large differences in the serum concentrations of ovarian hormones, such as estrogen and progesterone (Janse De Jonge, 2003; Oosthuyse and Bosch, 2010). In particular, estrogen promotes increased fat oxidation (Kendrick et al.,

1987), sparing muscle and hepatic glycogen (Kendrick et al., 1987; Lavoie et al., 1987), and increasing exercise time until exhaustion (Nicklas et al., 1989). The menstrual cycle can be divided into the early follicular phase (EF), where estrogen is low, and the late follicular phase (LF), where only estrogen is high, focusing on changes in estrogen concentrations (Janse De Jonge, 2003; Oosthuyse and Bosch, 2010). Several studies have identified differences in substrate utilization during rest, exercise, and recovery across different menstrual cycle phases (Nicklas et al., 1989; Hackney, 1990; D'Eon et al., 2002). To understand the effect of milk intake in the recovery of women, it is necessary to consider the menstrual cycle and investigate the changes in the phases; however, the relationship between milk intake after exercise and the menstrual cycle remains known.

Therefore, this study aimed to examine the effects of the change in substrate and performance test when consuming carbohydrate beverage (CHO) or co-ingestion of carbohydrate with milk (MILK) during 4-h post-exercise recovery considering the menstrual cycle. We hypothesized that in CHO and MILK; LF resulted in a faster substrate recovery than EF and improved subsequent performance. Moreover, MILK intake at EF and LF resulted in a faster substrate recovery and improved post-recovery performance compared to CHO intake at EF and LF.

Methods

Participants

This study included 12 recreationally-active women (≥ 240 min·week⁻¹ of physical activity), having regular natural menstrual cycles 26 - 38 days in length with no oral contraceptives use for a minimum of 6 months prior to study inclusion (height: 1.59 ± 0.55 m; body weight: 55.1 ± 6.0 kg; body mass index: 21.7 ± 1.9 kg·m⁻²; maximal oxygen uptake [VO_{2max}]: 37.9 ± 5.0 mL·kg⁻¹·min⁻¹; and maximal workload capacity [W_{max}]: 172.9 ± 27.9 W). The participants had no history of smoking and chronic diseases. All experimental procedures were conducted in accordance with the Declaration of Helsinki and were approved by the ethics committee of Nippon Sport Science University (No. 020-H045, date of approval; August 25, 2020). The study purpose and experimental procedures were explained to all participants prior to their participation in this study, and all participants provided written informed consent for participation in the study.

The participants wrote a menstrual cycle diary for three consecutive cycles to determine the average cycle length, which was calculated as the number of days between the onsets of consecutive menses. The menstrual diary determined the approximate EF and estimated day of ovulation, LF. The participants used an ovulation predictor kit for luteinizing hormone surge detection (DO-TEST; ROHTO Pharmaceutical Co., Ltd, Tokyo, Japan) during the experimental cycle to verify each menstrual cycle phase. This kit was instructed to follow the manufacturer's instructions to perform ovulation prediction testing for 7 consecutive days during one cycle. The results were confirmed by visual inspection of the test strip (participant) and photographic records (experiment staff). Additionally, serum estradiol and progesterone concentrations were

measured to determine each phase (Janse DE Jonge, Thompson and Han, 2019).

Preliminary testing

W_{max} and VO_{2max} of the participants were determined by performing an incremental exercise test using a cycle ergometer (Corival1000S; Lode B. V. Medical Technology, Groningen, The Netherlands). The incremental exercise test was started at 75 W, which was increased by 25 W every 3 min until complete exhaustion (Céline et al., 2011). The participants maintained a pedal speed of 60 rpm on an electronic metronome during the exercise. The determination criteria for all out at two or more points were as follows: the participants could no longer maintain the specified pedal speed of 60 rpm; the rating for perceived exertion reached 20; the respiratory exchange ratio (RER) exceeded 1.2; and the participants almost reached the maximum heart rate estimated for age (i.e., $220 - \text{age} \pm 5$ beats/min). W_{max} and VO_{2max} were mostly unchanged by the menstrual cycle (Redman, Scroop and Norman, 2003; Smekal et al., 2007; Tsampoukos et al., 2010); therefore, the W_{max} and VO_{2max} were randomly measured in each menstrual cycle phase.

Experimental protocols

Our test was performed in four randomized crossover trials in EF + CHO, LF + CHO, EF + MILK, and LF + MILK. The participants arrived at the laboratory of the university at 0900. Muscle and liver glycogen depletion was established by performing an intense exercise protocol using a cycle ergometer (exercise one) (Figure 1). The exercise protocol started with a 10-min warm-up at 50% W_{max}. Thereafter, the participants cycled for a 2-min block period at alternating workloads of 90% and 50% W_{max}. This continued until the participants were no longer able to complete a 2-min exercise period at 90% W_{max} with a cycling cadence of 60 rpm. At this point, the high-intensity blocks were reduced to 80% W_{max}, and the same regimen was continued. When the participants were no longer able to complete a 2-min block period at 80% W_{max}, the exercise intensity of the blocks was further decreased to 70% W_{max}. The participants were allowed to stop when the pedaling speed could not be maintained at 70% W_{max}. The exercise intensity was reduced based on the rate of perceived exertion, heart rate, and pedal speed (60 rpm). Each session was terminated when the participant could no longer maintain a cadence of 60 rpm for 15 s. This protocol has often been used in previous studies as a muscle and liver glycogen depletion protocol (Fuchs et al., 2016; Trommelen et al., 2016). Carbohydrates are used during prolonged moderate-to-high intensity exercise, and glycogen stored in muscle and liver may deplete with longer exercise durations (Bergström et al., 1967; Romijn et al., 1993). Therefore, this exercise protocol and intensity was chosen for this study. The participants were observed for the next 240 min, during which the beverage was received with CHO or MILK every 30 min until 210-min post-exercise. Blood samples were measured, and gas samples were collected before the exercise (PRE), immediately after exercise (POST0), 120 min post-exercise (POST120), and 240 min post - exercise (POST240). The cycling time to fatigue at 90%W_{max} was assessed using an exercise capacity test (exercise two).

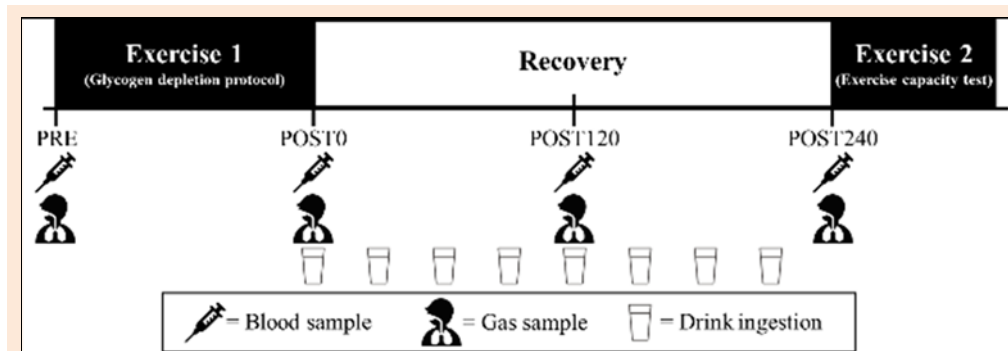


Figure 1. A schematic representation of the experiment. PRE, before exercise; POST0, immediately after exercise; POST120, 120 min post-exercise; POST240, 240 min post-exercise.

Diet and recovery beverage

All participants were instructed to maintain their normal diet as constant as possible during the experiment. Additionally, the participants recorded their food intake for 2 days prior to each trial. They abstained from meals 12 h prior and from alcohol and caffeine 24 h prior to each experiment. Water was provided ad libitum on each test day (1.0–2.0 L). However, the participants consumed the same amount of water on all test days. The intake of energy and macronutrient content of food and beverage items consumed for 2 days prior to each trial was analyzed using a nutrition analysis software (Excel Eiyokun, Kenpakusya, Tokyo, Japan) by a registered dietitian.

The two beverages were CHO, which was prepared by dissolving carbohydrate (KONA-AME for Athletes, HABA Laboratories Inc., Tokyo, Japan) in 200 mL of sports beverage (containing 0 g of protein, 0 g of fat, and 6.2 g of carbohydrate in 100 mL; Pocari Sweat, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and MILK, which was prepared by dissolving carbohydrate in 200 mL of milk (containing 3.7 g of protein, 1.9 g of fat, and 5.4 g of carbohydrate in 100 mL; Meiji Oishii Teishibounyu, Meiji Co., Ltd., Tokyo, Japan). The rate of carbohydrate intake in CHO was $1.3 \text{ g} \cdot \text{kg} \text{ BM}^{-1} \cdot \text{h}^{-1}$, whereas MILK provided $1.0 \text{ g} \cdot \text{kg} \text{ BM}^{-1} \cdot \text{h}^{-1}$ of carbohydrate. MILK and CHO are isocaloric. These doses were based on previous findings that 1.0–1.2 g carbohydrate per kg body weight maximized muscle glycogen storage during short-term recovery after exercise, and additional carbohydrate more than 1.2 g per kg body weight will not further increase this glycogenic response (Alghannam et al., 2018). Participants were instructed to drink the amount provided within 5 min.

Blood sample analysis

Blood samples from the antecubital vein were collected in 8 mL serum separation tubes. These samples were left to clot at room temperature before being centrifuged at 3,000 rpm for 10 min at 4°C. Serum was obtained from all blood samples and analyzed for concentrations of estradiol, progesterone, free fatty acids (FFA), and insulin. Serum estradiol and progesterone levels were analyzed using a chemiluminescent immunoassay method with kits (Abbott Japan Co., Ltd., Tokyo, Japan) [estradiol: detection range of 10–1,000 pg/mL, coefficient of variation (CV): < 7%; progesterone: detection range of 0.1–40.0 ng/mL, CV: < 10%]. FFA concentrations were measured by employing an ultraviolet method using kits (FUJIFILM Wako Pure Chemical

Corporation, Osaka, Japan) (detection range: 0.01–4.00 mEq/L, CV: < 1.5%). Furthermore, serum samples were analyzed for insulin using chemiluminescent immunoassay with kits (Abbott Japan Co., Ltd., Tokyo, Japan) (detection range of > 0.5 $\mu\text{U}/\text{mL}$, CV: < 2.5%). Lactate in whole blood was measured using a portable blood lactate analyzer (Lactate Pro2; Arkray, Tokyo, Japan) (detection range: 20–600 mg/dL, CV: < 2.8%). Blood glucose was determined using a glucose analyzer (Glucocard Diameter-alpha GT-1661; Arkray, Kyoto, Japan) (detection range: 0.5–25.0 mmol/L, CV: < 4.0%).

Gas analysis

RER, carbohydrates, and fat oxidation were measured using an expired gas analyzer (AE310-S Aero monitor; Minato Medical Science, Osaka, Japan) and breath-by-breath method. After 2 min of breathing equilibration, the average values for oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were calculated from 3 min of gas collection at each time period (Qin et al., 2017). The oxidation of carbohydrates [1] and fats [2] was calculated from the gas analysis results with the following formula (Jeukendrup and Wallis, 2005):

$$[1] = 4.585 \text{ VCO}_2 - 3.226 \text{ VO}_2$$

$$[2] = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

Statistical analyses

Sample size estimation was based on previous data, and a moderate effect size was calculated using G*power 3.1.9.4. A sample size of nine in a crossover design would provide a statistical power above 95% with an α -level of 0.05. Therefore, we recruited 12 participants to ensure adequate power and ample data sets. The experimental days and serum estradiol and progesterone concentrations at PRE were analyzed using the Friedman test to assess differences in menstrual cycle and recovery beverages as a factor. If the result was significant, the Wilcoxon signed rank tests were performed with a Bonferroni adjustment applied. Two days of food recording data, the area under the curve (AUC) of serum FFA concentrations, exercise time of the first exercise, and exercise capacity test were analyzed using one-way repeated-measures analysis of variance with menstrual cycle and recovery beverages as factors. Blood glucose, blood lactate, serum FFA, and serum insulin concentrations were analyzed using a two-way repeated-measures

analysis of variance with trial \times time as factors. When a significant main effect or interaction was identified, data were subsequently examined using the Bonferroni post-hoc test. The Pearson correlation coefficients were used to determine the association between the AUC of serum FFA concentrations during recovery and exercise capacity tests. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 28.0 (IBM, Armonk, NY, USA), and all data were reported as mean \pm standard deviation.

Results

Experiment day and ovarian hormones

The experimental days of LF + CHO and LF + MILK were significantly longer than those of EF + CHO (vs. LF + CHO, $p < 0.01$; vs. LF + MILK, $p < 0.01$) and EF + MILK (vs. LF + CHO, $p < 0.01$; vs. LF + MILK, $p < 0.01$, Table 1). However, no significant difference was found between the experimental days of EF + CHO and EF + MILK and those of LF + CHO and LF + MILK ($p = 0.15$) (Table 1).

Serum estradiol concentrations at PRE were significantly higher in LF + CHO and LF + MILK than those in EF + CHO (vs. LF + CHO, $p < 0.01$; vs. LF + MILK, $p < 0.01$) and EF + MILK (vs. LF + CHO, $p < 0.01$; vs. LF + MILK, $p < 0.01$). Serum progesterone concentrations at PRE were not significant in EF + CHO, LF + CHO, EF + MILK, and LF + MILK ($p = 0.21$).

Diet for two days before the experiment

The intake of energy did not significantly differ between EF + CHO, LF + CHO, EF + MILK, and LF + MILK (EF + CHO, 1876.3 ± 346.9 vs. LF + CHO, 1673.2 ± 490.0 vs. EF + MILK, 1733.0 ± 605.3 vs. LF + MILK, 1732.8 ± 616.8 kcal/day, $p = 0.64$). Protein, fat, and carbohydrate intake ratios did not significantly differ between EF + CHO, LF + CHO, EF + MILK, and LF + MILK (protein: EF + CHO, 63.4 ± 13.3 vs. LF + CHO, 60.0 ± 20.4 vs. EF + MILK, 62.6 ± 28.1 vs. LF + MILK, 63.1 ± 25.0 ; fat: EF + CHO, 70.6 ± 21.6 vs. LF + CHO, 63.7 ± 29.7 vs. EF + MILK, 67.5 ± 26.7 vs. LF + MILK, 67.2 ± 36.2 ; carbohydrate: EF + CHO, 243.8 ± 44.0 vs. LF + CHO, 214.8 ± 62.3 vs. EF + MILK, 216.9 ± 71.9 vs. LF + MILK, 214.1 ± 59.1 g/day, protein: $p = 0.97$; fat: $p = 0.92$; carbohydrate: $p = 0.38$).

Exercise capacity

The total exercise time until exhaustion at glycogen-depleting exercise (exercise one) were not significantly different in terms of menstrual cycle phases and recovery beverages (EF + CHO, 74.3 ± 14.8 vs. LF + CHO, 69.6 ± 26.2 vs. EF + MILK, 70.7 ± 20.2 vs. LF + MILK, 64.3 ± 19.6 min, $p = 0.35$). Moreover, the exercise time at exercise capacity test (exercise two) was not significantly different in terms of menstrual cycle phases and recovery beverages (EF + CHO, 3.1 ± 0.9 vs. LF + CHO, 3.0 ± 1.2 vs. EF + MILK, 3.2 ± 1.3 vs. LF + MILK, 3.2 ± 1.2 min, $p = 0.82$) (Figure 2). However, there was a significant positive correlation between the exercise time of the exercise capacity test and the AUC of serum FFA concentrations from POST0 to POST240 (EF + CHO, $r = 0.69$, $p < 0.05$; LF + CHO, $r =$

0.59 , $p < 0.05$; EF + MILK, $r = 0.83$, $p < 0.01$; LF + MILK, $r = 0.65$, $p < 0.05$).

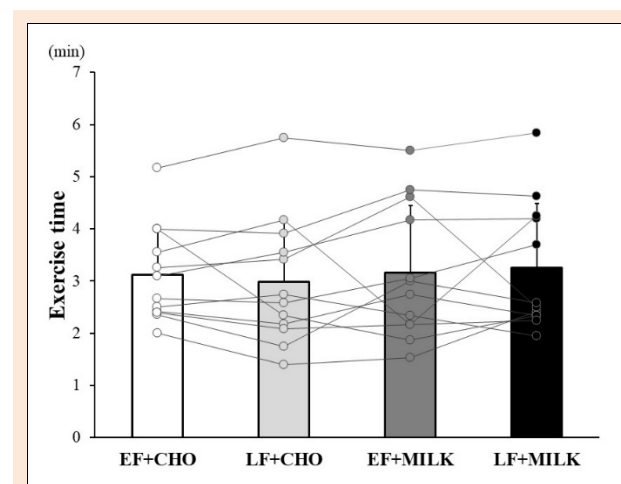


Figure 2. Exercise time of the exercise capacity test. Values are mean \pm SD. EF, early follicular phase; LF, late follicular phase; CHO, carbohydrate beverage; MILK, co-ingestion of carbohydrate with milk.

Substrates utilization and hormone concentrations

No significant differences in blood glucose, blood lactate, serum FFA, and serum insulin concentrations at PRE, POST0, POST120, POST240 were observed between EF + CHO, LF + CHO, EF + MILK, and LF + MILK (blood glucose, trial: $p < 0.05$; time: $p < 0.01$; trial \times time: $p = 0.55$; blood lactate, trial: $p = 0.20$; time: $p < 0.01$; trial \times time: $p = 0.06$; serum FFA, trial: $p = 0.80$; time: $p < 0.01$; trial \times time: $p = 0.11$; serum insulin, trial: $p = 0.17$; time: $p < 0.01$; trial \times time: $p = 0.64$) (Table 2). However, the AUC of serum FFA concentrations from POST120 to POST240 was significantly greater in EF + MILK and LF + MILK than in EF + CHO (vs. EF + MILK, $p < 0.05$; vs. LF + MILK, $p < 0.05$) and LF + CHO (vs. EF + MILK, $p < 0.01$; vs. LF + MILK, $p < 0.01$) (Figure 3).

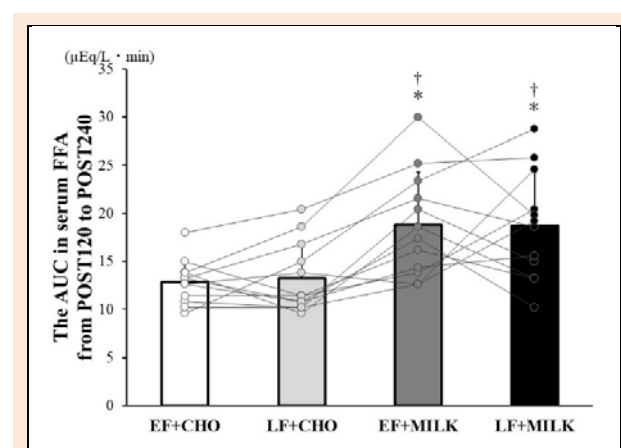


Figure 3. Area under curve of serum free fatty acids from 120 min post-exercise to 240 min post-exercise. Values are mean \pm SD. AUC, area under curve; FFA, free fatty acids; EF, early follicular phase; LF, late follicular phase; CHO, carbohydrate beverage; MILK, co-ingestion of carbohydrate with milk; POST120, 120 min post-exercise; POST240, 240 min post-exercise. * $p < 0.01$ vs. EF + CHO, † $p < 0.01$ vs. LF + CHO.

The RER at POST120 was significantly lower in EF + MILK and LF + MILK than in EF + CHO. Additionally,

the RER at POST120 showed a significantly lower trend in EF + MILK than that in LF + CHO (trial, $p = 0.23$; time, $p < 0.01$; trial \times time, $p < 0.05$). The average carbohydrate and fat oxidation at PRE, POST0, POST120, and POST240

were not significantly different between each menstrual cycle and beverage (carbohydrate, trial: $p = 0.79$; time: $p < 0.01$; trial \times time: $p = 0.38$; fat, trial: $p = 0.21$; time: $p < 0.01$; trial \times time: $p = 0.52$) (Table 2).

Table 1. Experiment day and ovarian hormone concentrations before exercise

	EF + CHO	LF + CHO	EF + MILK	LF + MILK
Experiment day	8.0 (3.8 – 8.0)	15.0 (12.3 – 16.0) *, †	6.0 (3.3– 7.8)	16.0 (14.3 – 18.5) *, †
Estradiol (pg/mL)	41.5 (34.5 – 49.0)	101.5 (63.8 – 286.5) *, †	38.5 (25.3 – 42.5)	139.5 (95.3 – 209.5) *, †
Progesterone (ng/mL)	0.2 (0.2 – 0.3)	0.3 (0.2 – 0.4)	0.2 (0.2 – 0.2)	0.4 (0.2 – 1.1)

Values are median (25 – 75%). EF, early follicular phase; LF, late follicular phase; CHO, carbohydrate beverage; MILK, co-ingestion of carbohydrate with milk. * $p < 0.01$ vs. EF + CHO; † $p < 0.01$ vs. EF + MILK.

Table 2. Substrate utilization and hormone concentrations.

	Trial	PRE	POST0	POST120	POST240
Blood glucose (mg/dL)	EF + CHO	86.2 \pm 5.4	96.2 \pm 12.6 **	99.8 \pm 11.1 **	74.3 \pm 17.3 *, ††, †††
	LF + CHO	82.8 \pm 5.8	90.4 \pm 11.9 **	90.7 \pm 15.4 **	73.0 \pm 12.5 *, ††, †††
	EF + MILK	85.8 \pm 5.3	95.6 \pm 15.1 **	96.5 \pm 14.8 **	81.1 \pm 15.2 *, ††, †††
	LF + MILK	85.4 \pm 7.2	101.3 \pm 16.1 **	99.8 \pm 16.0 **	78.9 \pm 9.3 *, ††, †††
Blood lactate (mmol/L)	EF + CHO	1.5 \pm 0.3	3.7 \pm 1.3 **	2.4 \pm 0.5 **, ††	2.3 \pm 0.4 **, ††, †††
	LF + CHO	1.4 \pm 0.2	5.0 \pm 2.8 **	2.3 \pm 0.6 **, ††	2.4 \pm 0.6 **, ††, †††
	EF + MILK	1.5 \pm 0.4	4.4 \pm 2.3 **	2.0 \pm 0.3 **, ††	1.9 \pm 0.4 **, ††, †††
	LF + MILK	1.4 \pm 0.3	4.6 \pm 2.9 **	1.8 \pm 0.4 **, ††	2.0 \pm 0.4 **, ††, †††
Serum free fatty acids (μ Eq/L)	EF + CHO	0.44 \pm 0.14	1.47 \pm 0.39 **	0.13 \pm 0.02 **, ††	0.08 \pm 0.03 **, ††, †††
	LF + CHO	0.53 \pm 0.19	1.45 \pm 0.45 **	0.13 \pm 0.04 **, ††	0.09 \pm 0.03 **, ††, †††
	EF + MILK	0.41 \pm 0.20	1.37 \pm 0.40 **	0.19 \pm 0.07 **, ††	0.13 \pm 0.03 **, ††, †††
	LF + MILK	0.54 \pm 0.24	1.36 \pm 0.39 **	0.18 \pm 0.06 **, ††	0.13 \pm 0.05 **, ††, †††
Serum insulin (μ U/mL)	EF + CHO	5.6 \pm 1.4	7.1 \pm 3.8	39.8 \pm 16.7 **, ††	36.7 \pm 21.9 **, ††
	LF + CHO	4.5 \pm 1.4	5.1 \pm 2.7	31.8 \pm 13.4 **, ††	34.1 \pm 20.5 **, ††
	EF + MILK	4.9 \pm 1.7	6.2 \pm 3.6	32.2 \pm 18.0 **, ††	34.3 \pm 20.2 **, ††
	LF + MILK	5.6 \pm 1.2	6.0 \pm 2.9	29.8 \pm 12.0 **, ††	29.8 \pm 19.0 **, ††
Respiratory exchange ratio	EF + CHO	0.77 \pm 0.05	0.81 \pm 0.05	0.82 \pm 0.02 *	0.86 \pm 0.04 **, ††, ††
	LF + CHO	0.74 \pm 0.05	0.80 \pm 0.07	0.80 \pm 0.04 *	0.85 \pm 0.05 **
	EF + MILK	0.77 \pm 0.05	0.79 \pm 0.04	0.77 \pm 0.03 §§,	0.85 \pm 0.04 **, ††, †††
	LF + MILK	0.74 \pm 0.04	0.81 \pm 0.05 **	0.78 \pm 0.03 §§	0.87 \pm 0.09 **, †††
Average carbohydrate oxidation (g/min)	EF + CHO	0.08 \pm 0.07	0.51 \pm 0.27 **	0.13 \pm 0.03 **, ††, †††	0.18 \pm 0.05 **, ††, †††
	LF + CHO	0.05 \pm 0.07	0.59 \pm 0.41 **	0.12 \pm 0.06 **, ††, †††	0.18 \pm 0.08 **, ††, †††
	EF + MILK	0.08 \pm 0.06	0.51 \pm 0.31 **	0.08 \pm 0.04 **, ††, †††	0.19 \pm 0.08 **, ††, †††
	LF + MILK	0.04 \pm 0.04	0.63 \pm 0.25 **	0.09 \pm 0.03 **, ††, †††	0.20 \pm 0.09 **, ††, †††
Average fat oxidation (g/min)	EF + CHO	0.09 \pm 0.02	0.38 \pm 0.21 **	0.08 \pm 0.02 ††	0.06 \pm 0.02 **, ††, †††
	LF + CHO	0.10 \pm 0.02	0.46 \pm 0.18 **	0.08 \pm 0.02 ††	0.06 \pm 0.02 **, ††, †††
	EF + MILK	0.09 \pm 0.02	0.47 \pm 0.18 **	0.10 \pm 0.02 ††	0.06 \pm 0.02 **, ††, †††
	LF + MILK	0.10 \pm 0.02	0.47 \pm 0.19 **	0.10 \pm 0.02 ††	0.06 \pm 0.02 **, ††, †††

Values are mean \pm SD. EF, early follicular phase; LF, late follicular phase; CHO, carbohydrate beverage; MILK, co-ingestion of carbohydrate with milk. PRE, before exercise; POST0, immediately after exercise; POST120, 120 min post-exercise; POST240, 240 min post-exercise. * $p < 0.05$ vs. PRE; ** $p < 0.01$ vs. PRE; † $p < 0.05$ vs. POST0; †† $p < 0.01$ vs. POST0; ††† $p < 0.05$ vs. POST120; †††† $p < 0.01$ vs. POST120; §§ $p < 0.01$ vs. EF + CHO, || $p < 0.1$ vs. LF + CHO.

Discussion

We aimed to investigate the recovery effect of the ingestion of MILK after exercise in active women and compared the change in substrate utilization and exercise capacity test when consuming CHO or MILK during a short-term (4-h) post-exercise considering the menstrual cycle.

The first result of our study showed that the menstrual cycle did not affect substrates and exercise capacity tests when CHO or MILK was ingested during recovery. Estradiol increases fat utilization, reduces muscle and hepatic glycogen utilization, and prolongs exercise time to fatigue (Kendrick et al., 1987; Lavoie et al., 1987; Nicklas et al., 1989; Hackney, 1990; D'Eon et al., 2002). Although changes in substrate utilization using blood samples have not been shown before and after post-exercise recovery, a previous study demonstrated that muscle glycogen concentrations significantly increased in the luteal phase (LP) with

high estrogen levels during the menstrual cycle compared to EF (Nicklas et al., 1989; McLay et al., 2007). Therefore, changes in ovarian hormones related to the menstrual cycle may also be associated with substrate utilization during recovery after exercise. Many studies have investigated the changes in ovarian hormones and menstrual cycles and the changes in substrate utilization and exercise capacity during rest and exercise; however, most have not examined the short recovery and/or changes in post-recovery performance. In a study in which muscle glycogen synthesis was increased by LP rather than FP during post-exercise recovery for 3 days, the percentage of dietary carbohydrate ingested by its participants was lower than that considered optimal for maximal glycogen resynthesize. However, no difference in muscle glycogen between EF and LP was observed 3 days after loading of equal energy carbohydrate (McLay et al., 2007). Regarding the acute recovery period, women in the EF of their menstrual cycle do not differ in

circulating substrates, such as blood glucose and lactate, and muscle glycogen synthesis when compared with males with low ovarian hormones after intake of matched amounts (per kilogram of body mass) of carbohydrate or a carbohydrate–protein beverage and food ingestion (Tarnopolsky et al., 1997). Furthermore, a study during exercise has reported that substrate metabolism and performance are influenced by the menstrual cycle phase, but glucose ingestion minimizes these effects (Campbell, et al., 2001). In this experiment, MILK and CHO intake conditions were based on how to maximize glycogen recovery during the recovery period, such as the amount of carbohydrates, exercise start time, and intake timing (Betts and Williams, 2010; Alghannam et al., 2018). Therefore, the effect of the menstrual cycle may be minimized if sufficient nutrients are available for substrate recovery during short recovery after exercise. Additionally, FFA concentrations increased for at least 3–6 h after the exercise (Magkos et al., 2009), and the changed transporters and insulin that affect substrate changes during post-exercise recovery (Holten et al., 2004). These changes were indicated by the effects of ovarian hormones, which change with the menstrual cycle, similar to those of the exercise, and the effects of exercise stimulation might be greater than the effects of estrogen. Therefore, our results suggest that there is no difference in the concentrations of circulating substrates, such as blood glucose, blood lactate, and serum FFA, hormone (serum insulin), RER, and carbohydrate and fat oxidation for MILK or CHO intake during recovery across different menstrual cycle phases because the influence of beverage intake and exercise is larger than the influence of the menstrual cycle during short recovery post-exercise.

The second result of our study showed the significant positive correlation between the exercise time of the exercise capacity test and the AUC of the serum FFA concentrations during recovery in all trials, especially in both phases of the menstrual cycle. In particular, the ingestion of MILK showed an increased AUC of FFA concentration from POST120 to POST240 than CHO. Therefore, increasing FFA concentrations during the short-term recovery can improve the exercise capacity after recovery, and FFA concentrations were increased more by ingesting MILK compared to CHO during recovery. Milk and dairy chocolate milk has been recently reported to be an effective post-exercise beverage because of its apparent ability to restore energy stores and enhance performance after a recovery period (Russo et al., 2019; James et al., 2019). These previous studies have shown that higher fat contents of milk and dairy chocolate milk compared with control beverages could have resulted in faster recovery and improved performance (Thomas et al., 2009). Previous studies have reported that muscle glycogen repletion has a high metabolic priority during recovery, and utilization of fat is essential to cover the energy expenditure in muscles (Brown et al., 2013; Lundsgaard et al., 2020). A sustained increase in FA oxidation during recovery may support the resynthesis of intramuscular triglycerides and the resynthesis of skeletal muscle glycogen stores from the available plasma glucose. Therefore, in the present study, higher fat oxidation and FFA concentrations during post-exercise recovery have al-

lowed for the resynthesis of some intramuscular triglycerides and the replenishment of muscle glycogen due to MILK intake. Additionally, previous studies investigating recovery and performance have reported that increased glycogen synthesis during recovery improves exercise performance after recovery (Bergström et al., 1967; Tsintzas et al., 1996; Alghannam et al., 2016b). Although there was no difference in post-recovery exercise capacity tests between MILK and CHO ingestion in this study, a positive correlation was found between the AUC of FFA concentrations during the recovery and exercise capacity tests. Our study suggests that increased FFA concentrations during recovery restored the energy substrate faster and improved subsequent exercise capacity tests, and MILK intake was superior to CHO intake in terms of further increasing FFA concentrations during recovery. However, exercise that uses fat primarily as an energy source is a low-to-moderate endurance exercise. In previous studies, exercise performance after recovery has often been measured using low-to-moderate exercise intensity. Whether MILK intake is effective for the recovery of higher intensity exercise, such as in our study, remains unknown. Therefore, further research is required.

Blood glucose, blood lactate, and serum insulin concentrations showed no significant differences at all trials when either CHO or MILK was ingested during recovery. Previous studies have reported that the augmented insulin concentrations decreased blood glucose and increased muscle glycogen synthesis following combined carbohydrate-protein ingestion than carbohydrate ingestion alone (Williams et al., 2003; Betts and Williams, 2010). These studies showed that the congestion of protein has no further effect on glycogen synthesis when protein has been included in solutions providing carbohydrate at a lower ingestion rate (i.e., ≤ 0.8 g/kg/h) and with adequate CHO intake (Betts and Williams, 2010; Burke, Van Loon and Hawley, 2017). Previous studies have shown the benefit of the combined carbohydrate-protein ingestion was due to the increase in available energy (Betts and Williams, 2010; Burke et al., 2017). This study was considered and performed to achieve the same effect of carbohydrate and the same energy intake between combined carbohydrate-protein ingestion and carbohydrate ingestion alone during recovery using MILK, which is easy for anyone to purchase and use. Therefore, the CHO and MILK trials had the amount of carbohydrate greater than 0.8 g/kg/h, which was adequate for carbohydrate intake during recovery. Our results showed the ingestion of MILK did not influence blood glucose, blood lactate, and serum insulin concentrations during recovery. However, as previously mentioned, the AUC of serum FFA concentrations during recovery was significantly different between CHO and MILK. Milk contains fat compared to CHO. Therefore, MILK ingestion may have increased circulating free fatty acids in the blood, suggesting that MILK ingestion during recovery led to a greater increase in serum FFA compared to CHO ingestion.

This study had some limitations. It was not possible to unify the types of CHO and/or adjust the amount of protein and fat because milk was used in this study. However, milk is easily available to anyone and is very convenient

for use in the field. Counterbalancing was not possible because a minimum of 24 participants was required for counterbalancing, although this experiment was performed with calculated sample size. However, the order of the four trials differed for all 12 participants in this study. Moreover, we conducted multiple regression analysis between the order of experiments and each index, such as exercise duration and FFA concentrations. The results showed no significant differences in the order of the experiments and each index; for example, the exercise time of the exercise capacity test ($P < 0.56$; the AUC of serum FFA concentrations from POST0 to POST240: $P < 0.73$). Therefore, we believe that the order of the experiments is unlikely to have affected FFA concentrations or exercise performance. The participants in this study were not athletes, unlike those in previous studies. The intensity of the exercise may have been too high for a recreationally-active woman. However, we selected women who regularly performed endurance exercises and have previously confirmed that the current protocol can be implemented (Matsuda et al., 2020). Furthermore, this study is important because it considers the menstrual cycle of women, investigates the effect of MILK intake during recovery, and can provide data for investigating efficient recovery methods for women. Future studies may further emphasize the results of this study by measuring muscle, liver glycogen, and blood.

Conclusion

We investigated the effect of recovery after exercise using milk, considering the menstrual cycle in active women. The results indicated that MILK and CHO were not significantly different in terms of substrate utilization during recovery and exercise capacity tests after recovery in different menstrual cycle phases. However, MILK increased the AUC of FFA concentrations from POST120 to POST240 compared to CHO, and increased AUC of FFA concentrations during recovery has improved exercise capacity. Our study shows that the menstrual cycle does not affect the post-exercise recovery of women who consume enough carbohydrates during recovery, but the recovery speed may be positively affected by MILK.

Acknowledgements

We thank all the participants who volunteered for this study, as well as the team staff. This study was supported by the Japan Milk Academic Alliance and JSPS KAKENHI Grant Number JP20J13547. The experiments complied with the current laws of the country in which they were performed. The authors declare no conflicts of interest. The datasets generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author, who was an organizer of the study.

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Key points

- MILK and CHO was not significantly different in substrate utilization during recovery and exercise capacity tests after recovery in the different menstrual cycle phases when women consume enough carbohydrates during recovery.
- MILK increased the AUC of FFA concentrations during recovery improved exercise capacity compared to CHO.
- Milk is an effective post-exercise beverage in healthy women.

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