Timing of post-exercise carbohydrate–protein supplementation: roles of gastrointestinal blood flow and mucosal cell damage on gastric emptying in humans

Running title: Gut blood flow and gastric motility after strenuous exercise

Authors:

Hideaki Kashima¹, Nao Harada¹, Kanae Miyamoto¹, Masaki Fujimoto¹, Chiaki Fujita¹, Masako (Yamaoka) Endo¹, Toshio Kobayashi², Akira Miura¹, and Yoshiyuki Fukuba¹

Affiliations:

¹Department of Exercise Science and Physiology, School of Health Sciences, Prefectural University of Hiroshima, 1-1-71 Ujina-higashi, Minami-ku, Hiroshima 734-8558, Japan

²Department of Health Promotion and Development, Graduate School of Health Sciences, Hiroshima University, 1-2-3 Kasumi Minami-ku, Hiroshima 734-8551, Japan
Corresponding author: Yoshiyuki Fukuba, Ph.D., FACSM; Telephone: +81-82-251-9806; Fax: +81-82-251-9806; E-mail: fukuba@pu-hiroshima.ac.jp

Role of each author in the work: All authors have approved the final version of the manuscript. H.K. and Y.F. contributed to the conception and design of the study; generation, collection, assembly, analysis, and interpretation of data; and drafting of the revision of the manuscript. N.H, K.M., M.F., C.F., and T.K. assisted in the collection, assembly, analysis, and interpretation of data. M.E. and A.M. assisted in the interpretation of data.

Word count for entire manuscript (excluding references): 4566 words

Total number of figures: 5
ABSTRACT:

It is well known that protein ingestion immediately after exercise greatly stimulates muscle protein synthesis during the post-exercise recovery phase. However, immediately after strenuous exercise, the gastrointestinal (GI) mucosa is frequently injured by hypoperfusion in the organ/tissue, possibly resulting in impaired GI function (e.g., gastric emptying; GE). The aim of this study was to examine the effect of GI blood flow on the GE rate. Eight healthy young subjects performed an intermittent supramaximal cycling exercise for 30 min, which consisted of a 120% \( \dot{VO}_2 \) peak for 20 s, followed by 20 W for 40 s. The subjects ingested 300 mL of a nutrient drink containing carbohydrate–protein at either 5 min post-exercise in one trial (PE-5) or 30 min post-exercise in another trial (PE-30). In the control trial (Con), the subjects ingested the same drink without exercise. The celiac artery blood flow (CABF) and superior mesenteric artery blood flow (SMABF) and GE rate were assessed by ultrasonography. Before drink ingestion in PE-5, CABF significantly decreased from baseline, whereas in PE-30, it returned to baseline. Following drink ingestion in PE-5, CABF did not change from baseline, but it significantly increased in PE-30 and Con. SMABF increased significantly later in PE-5 than in PE-30 and Con. The GE rate was consistently slower in PE-5 than in PE-30 and Con. In conclusion, the CABF response after exercise seems to modulate the subsequent GE rate and SMABF response.

Keywords: gastrointestinal blood flow, mucosal cells, gastric emptying rate, high-intensity
intermittent exercise

NEW & NOTEWORTHY:

A carbohydrate–protein drink was ingested at either 5 min (i.e., profoundly decreased celiac artery blood flow; CABF) or 30 min (i.e., already recovered CABF) post-exercise. In the 5 min post-exercise trial, the gastric emptying (GE) rate and superior mesenteric artery blood flow (SMABF) response were slower than those in the 30 min post-exercise trial. The GE rate and SMABF response may be altered depending on the post-exercise CABF response.
INTRODUCTION:

According to a new position paper, carbohydrate–protein supplementation during the post-exercise recovery period is a useful nutritional strategy for promoting muscle protein anabolism (42). In order to achieve maximum muscle adaptation to exercise, laboratory-based nutritional studies have demonstrated that “immediate” post-exercise consumption of protein can elicit greater muscle protein synthesis, whereas fasting for 2–3 h after exercise blunts this response (21, 25). Therefore, carbohydrate-protein supplementation as soon as possible (at least within 45 min) after endurance exercise has been recommended; this is also applicable in case of resistance exercise (16).

During and immediately after exercise, however, it is well recognized that 25%–70% of endurance athletes experience transient anorexia as well as gastrointestinal (GI) disorders such as nausea, vomiting, abdominal angina, and bloody diarrhea (29, 41, 43). These exercise-induced GI disorders can occur quite frequently, creating concern regarding whether they could lead to decreased exercise performance and subsequent recovery in athletes. Although there are numerous causes of exercise-induced GI disorders, some recent studies have considered the reduction of GI blood flow as one of the underlying mechanisms of GI disorders (5, 40, 43). Strenuous exercise with high intensity and/or prolonged duration often causes a reduction of celiac artery blood flow (CABF) (28), superior mesenteric artery blood flow (SMABF) (28, 30), as well as blood flow in the gastric mucosa (27, 39, 44, 46), portal vein...
(31), and whole viscera (26, 33), resulting in serious damage to small intestinal mucosal cells. This was concomitant with intestinal-fatty acid-binding protein (I-FABP) appearance in the circulating blood (2, 22, 44, 45, 46). In clinical practice, plasma I-FABP has been used as a marker for the diagnosis of hypoperfusion and ischemia in GI tract (4), leading to GI complaints (40, 43). In such situations, i.e., under ongoing hypoperfusion in the GI tract immediately after the cessation of exercise, the consumption of a nutrient beverage may result in impaired digestion and/or absorption. In this regard, van Wijck et al. (45) observed the appearance of plasma phenylalanine after ingestion of dietary protein (20 g) immediately after high-intensity resistance exercise. They showed that compared with a nonexercise trial, the plasma phenylalanine was lower for approximately 30 to 60 min during the post-exercise phase. They interpreted that the strenuous exercise-induced GI hypoperfusion damaged intestinal mucosal cells and consequently decreased plasma phenylalanine appearance rates (i.e., impaired GI function). GI blood flow was not measured. Therefore, the precise etiology from exercise-induced GI hypoperfusion to impaired GI function remains to be elucidated. In other words, the GI blood flow response and GI function after exercise should be clarified, with special attention being paid to the timing of nutrient supplementation.

Ingested foods temporarily remain in the stomach and are digested by acid and enzyme secretions. Digested food (i.e., chyme) is then emptied from the pyloric stomach to the intestinal duodenum and is digested and absorbed in the small intestine. During these
processes, gastric emptying (GE) is an important GI function that determines the digestive–absorptive rate of nutrients. Therefore, the GE rate has been often used as an index to evaluate fluid delivery during exercise because these data have provided useful information to prevent excessive dehydration and changes in electrolyte balance in athletes as well as to maintain blood glucose concentrations during exercise. Several previous reviews have summarized the effects of exercise intensity and duration time on GE rates during exercise (3, 14, 20, 24). The fluid GE rate is substantially unaffected or rather accelerated during mild to moderate exercise, in contrast to the delay observed during intensities greater than 70%–75% maximal oxygen consumption ($\dot{V}O_2\text{max}$) and high-intensity intermittent exercise (3, 14, 20, 24).

On the other hand, only limited data exist on the GE rate after exercise, despite the importance of promoting quick rehydration, refueling, and repair of damaged tissues from exercise (16, 23). Evans et al. (8) have recently reported the effects of high-intensity interval cycling exercises (10 sets of 1 min at peak power output) on the GE rate of a 5% glucose solution at 30 min after exercise. They found that the gastric half emptying time (a standard index of the GE rate) did not differ from that in the control group that did not engage in exercise. However, post-exercise GE regulation remains unclear when a nutrient beverage is ingested immediately after the cessation of exercise because, with such timing, dietary protein absorption is delayed by damaged intestinal mucosal cells (45). Post-exercise GE may therefore be affected by the timing of nutrient supplementation even in the post-exercise early phase,
such as immediately versus 30 min after exercise.

In this study, we, therefore, hypothesized that the GE rate is delayed when a post-exercise nutrient beverage is supplemented immediately after the cessation of exercise rather than 30 min later (8). This is based on research indicating that GI blood flow is markedly decreased immediately after exercise but gradually recovers to baseline levels (27, 39, 44, 45, 46). Accordingly, using the same type of exercise as that in a previous study (i.e., high-intensity interval cycling exercise) (8), we aimed to investigate the effect of the timing of post-exercise carbohydrate–protein supplementation on GE rates and its relation to GI blood flow, with a concomitant measure of the damage of GI mucosal cells.

MATERIALS & METHODS:

Subjects:

Eight healthy young subjects (3 females and 5 males; age, 22 ± 3 years; height, 166 ± 6 cm; weight, 56 ± 2 kg; mean ± SD) participated in this study. The subjects were normotensive, did not smoke or take any medication, and had no history of autonomic dysfunction or cardiovascular disease. The study protocol was performed in compliance with the Declaration of Helsinki and approved by the Ethics Committee of the Prefectural University of Hiroshima (approval number: HH007). All the participants provided written informed consent to participate before study commencement.
Preliminary test session:

To determine individual target work rates, a ramp incremental exercise test using a cycling ergometer (XL-75III, Combi Wellness Corp., Tokyo, Japan) was performed at least 1 week prior to the experiment. This test included 2 min of baseline rest in the upright position, followed by 2 min of baseline exercise at 20 W and incremental ramp exercise at 15–20 W·min\(^{-1}\) still the individual’s tolerance limit. The subjects were instructed to maintain pedal frequency at 60 rpm. The test was terminated when the subjects could not maintain 50 rpm despite maximal exertion. Individual target work rates at a 120% \(\dot{V}O_2\) peak were then determined (232 ± 36 W).

Main trial session:

The subjects arrived between 8:30 and 10:30 a.m. in the laboratory on trial days after having abstained from strenuous exercise and alcohol and caffeine for at least 1 day. All the subjects were randomly assigned to participate in three experimental protocols on separate days (at least 1 week apart). In the 4 h prior to each experimental trial, the subjects consumed a standardized semi-liquid diet of 400 kcal containing 79 g carbohydrate, 7.5 g protein, and 5.6 g fat. We confirmed that this beverage was completely emptied from the stomach at the onset of each experiment using ultrasonography, as described below. The subjects were seated in a
chair in a quiet room for 15 min, and the temperature and humidity were maintained at 23 ± 1°C and 40 ± 5%, respectively. The subjects performed a single bout of exercise consisting of a 2-min warm-up at 20 W, followed by 30 sets of high intensity interval exercises, in which 1 set consisted of 120% VO$_2$ peak for 20 s and 20 W for 40 s. Subjects ingested 300 mL of a nutrient drink containing 9 g carbohydrate and 15 g whey protein (ZAVAS Protein Drink, Meiji Co. Ltd. Tokyo, Japan) at either 5 min post-exercise in one trial (PE-5) or 30 min post-exercise in another trial (PE-30) and rested for 60 min. In the control trial (Con), the subjects ingested the same nutrient drink without engaging in exercise.

Measurements:

The heart rate (HR) was continuously monitored using an electrocardiogram (DINASCOPE DS8100 System, Fukuda Denshi Co., Ltd, Tokyo, Japan) throughout the protocol. With the exception of the exercise phase, the mean blood velocity (MBV) and vessel diameter of the celiac artery (CA) and superior mesenteric artery (SMA) were recorded during the trial to evaluate the blood flow of each artery (i.e., CABF and SMABF) using pulsed Doppler ultrasound sonography (LOGIQ S6, GE Medical Systems, Tokyo, Japan) with a 3.5-MHz convex probe. Measurements were performed within 1–1.5 cm of the original...
branch of each artery. After adjustment of the sample volume width to cover the arterial
diameter during the expiratory phase, the Doppler probe was kept constant on the subject’s
skin surface. CA supplies blood to the stomach, pancreas, spleen, and liver (i.e., upper GI and
other areas), and SMA mainly supplies blood to the jejunum and ileum (i.e., small intestinal
areas). CABF and SMABF remarkably increased after macronutrient food consumption (17,
37), and such responses support the various functions of the GI system (e.g., motility and
delivery of absorbed nutrients and secretion of gut hormones). Based on anatomical and
physiological characteristics, it is preferable to simultaneously measure and evaluate both
CABF and SMBF because the activation of the upper GI and small intestinal areas can be
assessed separately. The Doppler beam insonation angle was maintained at ≤60° relative to
the artery. According to methods established in our laboratory (6, 7, 17), the Doppler signals
for antegrade and retrograde flows and the electrocardiogram signal were digitally sampled
online at 20 kHz using an A/D converter (PowerLab 8/30, ADInstruments, Colorado Springs,
CO, USA) and then analyzed offline by Doppler signal processing software (fast Fourier
transform analysis) to calculate second-by-second MBV. The vessel diameter was obtained by
analyzing pictures of the vertical section of blood vessels using B-mode ultrasound. BF was
calculated as follows: BF (mL/min) = MBV (cm/s) × [vessel diameter (cm)/2]² × π × 60 (s).
In this study, GE was evaluated from the change in cross-sectional area (CSA) of the
gastric antrum before and after ingestion of a nutrient drink using ultrasonography, which
provided a noninvasive method in real time. This method has been previously confirmed to closely correlate with the scintigraphy method, which is considered the gold standard for GE assessment (15). The cross-sectional image of the gastric antrum was displayed with the left lobe of the liver, superior mesenteric vein, and abdominal aorta in a longitudinal section as reference markers according to the standard measuring method using ultrasound sonography (LOGIQ S6, GE Medical Systems, Tokyo, Japan) with a convex probe (3.5 MHz). Because the peristaltic contractions in the gastric antrum could be observed periodically, imaging of the maximal antral dilatation was obtained. CSA of the gastric antrum was determined by tracing the outer layer of the gastric wall (serosa) according to the method used in a previous study (19). CSA of the gastric antrum immediately before (i.e., t = 0) and after (i.e., t = 5) ingestion of the nutrient drink was defined as 0% and 100%, respectively. The relative percent reduction in CSA at every time point was represented and calculated as % GE (t) \[\left(\frac{A_{(\text{post-}t)} - A_{(\text{immediately before})}}{A_{(\text{immediately after})} - A_{(\text{immediately before})}}\right) \times 100\]. In Con, MBVs in CA and SMA and CSA of the gastric antrum were measured at baseline and every 15 min after ingestion of the nutrient drink. In PE-5 and PE-30, these outcomes were additionally measured immediately and 25 min (only PE-30) after exercise. MBVs in CA and SMA and CSA of the gastric antrum were measured in this order every 1.5 min, 1.5 min, and 2 min, respectively. HR values were determined at 15-min intervals after ingestion of the nutrient drink. In PE-5 and PE-30, they were also determined at 15 min after exercise, during exercise, immediately
after exercise, and 25 min after exercise (only PE-30).

Blood sampling:

To quantitatively assess the GI cell damage following exercise, the plasma I-FABP level was measured. I-FABP is a well-established biochemical marker for early intestinal cell damage, and its levels elevate rapidly after episodes of acute intestinal ischemia/reperfusion (e.g., during and after heavy exercise) (2, 4, 22, 44, 45, 46). Capillary blood samples were collected by a finger prick on the right index and middle fingers at baseline and at 30 and 60 min after ingestion of the nutrient drink. In PE-5 and PE-30, blood samples were also collected immediately and 25 min (only PE-30) after exercise. To obtain plasma blood samples, capillary blood samples were collected in three post-heparin 75-µL capillary tubes and then centrifuged at 1500 ×g for 15 min at 4°C. Plasma samples were refrigerated at −80°C. Plasma I-FABP was analyzed using the ELISA Kit (Hycult biotechnology, Uden, the Netherlands, measurable concentration range = 47–3,000 pg/mL).

Subjective average appetite score:

Before and immediately after exercise as well as immediately before exercise and every 30 min later, the subject’s motivation to eat was assessed using 100-mm visual analogue scales. These were included four questions (hunger, fullness, desire to eat, and prospective
consumption). The scales for hunger, desire to eat, and prospective consumption ranged from 0 mm (most anorexigenic feeling) to +100 mm (most orexigenic feeling), whereas that for fullness ranged from 0 mm (“not full at all”) to +100 mm (“very full”). Using the scores of these four questions, a subjective average appetite score was calculated for each measurement time point according to the following formula (1, 34):

subjective average appetite score (mm) = \[\text{desire to eat} + \text{hunger} + (100 - \text{fullness}) + \text{prospective consumption}\]/4.

Data analysis:

Data are expressed as mean and standard error of mean. The effects of time and treatment on HR, GE rate, CABF, SMABF, I-FABP, and subjective average appetite scores were tested by two-way repeated analysis of variance (ANOVA). When a significant effect was detected, Dunnett’s and Tukey’s post-hoc tests were conducted to reveal effects of time (the change from baseline) and treatments, respectively. The statistical significance level was set at \( p \leq 0.05 \). All statistical analyses were performed with SPSS PASW version 18 (SPSS Inc., Chicago, Illinois, USA).

RESULTS:
**HR response:**

Baseline HR values did not show significant differences among the trials (p > 0.05) (Fig. 2). In PE-5 and PE-30, HR responses during and immediately after exercise were identical. After ingestion of the nutrient drink, among all the trials, HR values were the highest in the PE-5 trial, whereas HR values were higher in PE-30 than in Con.

Place Fig. 2 here.

**Plasma I-FABP response:**

There were no differences in baseline values of I-FABP between the exercise trials (PE-5: 207 ± 38 pg/mL, PE-30: 220 ± 31 pg/mL, p > 0.05) (Fig. 3). In PE-5, a significant increase was observed from baseline to immediately after exercise (t = −5) and 30 min after ingestion of the nutrient drink. In PE-30, a significant increase was observed from baseline to immediately (t = −30) and 25 min (t = −5) after exercise and 30 min after ingestion of the nutrient drink. No differences were observed between the exercise trials.

Place Fig. 3 here.

**GI blood flow responses:**

Baseline values of CABF did not differ among the three trials (Con: 329 ± 36 mL/min, PE-5: 330 ± 29 mL/min, PE-30: 322 ± 28 mL/min) (Fig. 4). In Con, CABF significantly
increased from baseline to 30 min after ingestion of the nutrient drink. In PE-5, CABF significantly decreased from baseline to immediately \((t = -5)\) after exercise and then returned to the baseline value but did not increase further after ingestion of the nutrient drink. In PE-30, CABF significantly decreased from baseline to immediately \((t = -30)\) after exercise, returned to the baseline value at 25 min \((t = -5)\) after exercise, and further increased significantly at 15 to 30 min after ingestion of the nutrient drink. At 15 min after ingestion of the nutrient drink, PE-30 showed a higher CABF response than PE-5.

Baseline values of SMABF did not differ among the three trials (Con: 329 ± 36 mL/min, PE-5: 330 ± 29 mL/min, PE-30: 322 ± 28 mL/min) (Fig. 4). In Con, SMABF significantly increased from baseline to 30 to 60 min but did not show a significant response at 45 min \((p = 0.054)\) after ingestion of the nutrient drink. In PE-5, SMABF did not significantly decrease from baseline to immediately \((t = -5)\) after exercise and then increased significantly at 30 to 60 min after ingestion of the nutrient drink. In PE-30, SMABF did not decrease from baseline to immediately \((t = -30)\) after exercise and then increased significantly at 15 to 60 min after ingestion of the nutrient drink. At 15 min after ingestion of the nutrient drink, PE-30 showed a higher SMABF response than PE-5.

Place Fig. 4 here.
Immediately before and immediately after ingestion of the nutrient drink, CSA of the gastric antrum did not differ among the trials. At 10 to 60 min after ingestion of the nutrient drink, relative % GE values for PE-5 were higher (i.e., the GE rate was slower) than those for Con (Fig. 5). At 10 to 45 min after ingestion of the drink, % GE values for PE-5 were also higher than those for PE-30. % GE for PE-30 was almost comparable to that for Con.

Place Fig. 5 here.

Subjective average appetite score:

Baseline values of subjective average appetite scores did not differ among the three trials (Con: 74.9 ± 5.8 mm, PE-5: 69.9 ± 5.4 mm, PE-30: 71.2 ± 6.1 mm). In PE-5 and PE-30, subjective average appetite scores significantly decreased from baseline to immediately after exercise (PE-5: 55.2 ± 5.1 mm, PE-30: 54.5 ± 5.1 mm). Following ingestion of the nutrient drink, significant changes from baseline were not observed for any of the trials (p > 0.05). No differences were noticed among the three trials following drink ingestion.

DISCUSSION:

This is the first study to investigate the effect of GI blood flow on the GE rate following strenuous exercise in humans. In this study, there were three key findings. First, acute mucosal damage of the small intestine itself does not directly affect subsequent GE rates.
Second, when CABF was still reduced immediately after exercise (i.e., in PE-5), the GE rate was delayed. As a result, the SMABF response was also delayed. Third, when the CABF returned to the resting baseline value 30 min after exercise (i.e., in PE-30), GE rates were comparable with those for Con. Consequently, the GE rate was altered by the timing of dietary supplementation after strenuous exercise, which may be partly affected by CABF responses irrespective of mucosal damage of the small intestine.

I-FABP significantly increased after supramaximal interval exercise for 30 min in all the subjects. The peak value of I-FABP was very similar to those in previous studies in which high-intensity cycling exercise was performed for 60 min at 70% of the maximal workload (44, 46). Therefore, the high-intensity interval exercise in the present study may have caused greater damage to small mucosal cells than prolonged endurance exercise during a short period of time. Previous trials have showed that exercise-induced hypoperfusion in the GI tract triggers acute damage to small intestinal mucosal cells (44, 45, 46). The I-FABP response was equal in PE-5 and PE-30, while GE rates were different. Therefore, we can conclude that acute mucosal damage of the small intestine itself does not directly affect subsequent GE rates.

The major finding in the present study was that when CABF decreased immediately after strenuous exercise (i.e., PE-5), the subsequent GE rate, especially from 0 to 30 min following the ingestion of the drink, was strongly suppressed. This acute suppression of GE rate immediately after exercise may be a key factor in determining subsequent digestive and
absorptive rates because the slope for CSA of the gastric antrum is similar at 30 and 60 min following the ingestion of the drink among the three trials. In contrast, when CABF recovered to baseline values after exercise (i.e., PE-30), the subsequent GE rate was unaffected. The latter result is consistent with that of Evans et al. (8). They reported that GE rates following the ingestion of carbohydrate beverage (595 mL of 5% glucose solution) at 30 min after high-intensity interval exercise were not different from those in a resting trial without exercise. Collectively, the results of this study and that of Evans et al. (8) suggest that GE rates are delayed immediately after high-intensity interval exercise and are gradually recovered toward resting baseline levels.

CA supplies blood to the stomach, pancreas, spleen, and liver, and this blood flow plays a certain role in supporting the maintenance of digestive activities (11). Therefore, the decreased CABF (i.e., hypoperfusion) following exercise may acutely suppress the GE rate. However, we did not directly measure the downstream arteries associated with gastric function, which represents a study limitation. To evaluate the gastric mucosal blood flow, previous studies have used gastric tonometry (26, 39, 44, 46). Even 50 min after 60 min of strenuous exercise, the gastric mucosal blood flow remained lowered (26, 44, 46). In addition, hypoperfusion followed by reperfusion in GI areas may be one of the factors modulating the GE rate after strenuous exercise. In fact, experimental ischemia–reperfusion of CA acutely suppresses the GE rate after food ingestion in rats (38). This suppression is due to the
disruption of the interstitial cell Cajal network (i.e., pacemaker cells), which originates from GI motility, and due to the induction of n-NOS production, which is related to the regulation of gastric motility via gastric mucosal damage following acute ischemia–reperfusion (38). In PE-5, the acute suppression of the GE rate may have therefore been induced not only by GI hypoperfusion but also by reperfusion after strenuous exercise.

SMABF had already returned to baseline values immediately after exercise in both exercise trials. Nevertheless, in PE-5, the SMABF response during recovery was slower than that in PE-30. This result can be explained by differences in the GE rate between the exercise trials because in previous research, a postprandial hyperemic response in SMA was observed when a digested meal (i.e., chyme) reached the small intestine from the stomach (11). Indeed, the SMABF response has a strong correlation with the GE rate (36). Although the SMABF response seems to reflect both digestive and absorptive activations, we did not evaluate the absorptive rate of nutrients (e.g., rates of blood glucose and amino acid appearance in circulating blood). In addition to the GE rate and GI blood flow, future research should therefore examine biomarker(s) to simultaneously evaluate the absorptive process.

Multiple physiological factors modulate the GE rate. GI hormones such as ghrelin, glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) have been well established to modulate the GE rate (14, 35). GLP-1 and PYY act to delay the GE rate, and both hormonal responses increase after exercise (14), whereas ghrelin accelerates the GE rate and decreases
after exercise (14). The present study did not measure these GI hormones. However, the subjective average appetite score may provide useful information to understand GI hormonal responses. Ghrelin has orexigenic effects, and GLP-1 and PYY have anorexigenic effects (12, 35). Only immediately after exercise, subjective average appetite scores decreased significantly from baseline in both the exercise trials. At this time point, a decrease in ghrelin and/or an increase GLP-1 and PYY may therefore be assumed and consequently result in acute suppression of the GE rate in PE-5. The relative dominance of sympathetic/parasympathetic activity may have also affected the GE rate in the present study. In previous research, mental stress and pain and/or cold stimulation of the hand, which can activate the sympathetic nervous system (13, 47), has been found to suppress the GE rate (9, 32) and impair gastric accommodation (10). PE-5 seems to have been the highest sympathetic activation among the three trials immediately before and after ingestion of the nutrient drink because at these time points, decreased CABF (i.e., sympathetic vasoconstriction) was observed, similar to the results of previous studies (28, 49). In addition, post-exercise parasympathetic activation may partly contribute to GE regulation. Mild-to-moderate-intensity exercise itself enhances subsequent gastric motility in healthy humans (18) and rats (48), and this enhanced response is absent in vagotomized rats (48). In the present study, following ingestion of the nutrient drink, a greater HR was observed in PE-5 than in PE-30 and Con. Thus, the parasympathetic activation in PE-5 seems to be lower
Dietary supplementation immediately after exercise is an accepted nutritional strategy to optimize exercise-induced muscular adaptations, facilitate the repair of damaged tissue, and restore water and nutrient loss (16). However, from the viewpoint of digestive–absorptive functions, our data imply that carbohydrate–protein supplementation immediately after strenuous exercise should not be recommended for athletes or training enthusiasts. In support of this concept, van Wijck et al. (45) recently have reported that protein supplementation immediately after high-intensity resistance exercise impairs digestion–absorption kinetics in dietary proteins during the recovery phase. The present data provide useful information to prevent GI distress and encourage effective recovery from physical fatigue among athletes. In summary, the CABF response after exercise impacts the subsequent GE rate and SMABF response, which may have implications for research on the timing of dietary supplementation after exercise.
GRANTS:

This study was supported in part by the Japan Society for the Promotion of Science KAKENHI (grant no. 40714746 to H.K.).

DISCLOSURES:

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES:


15. **Hveem K, Jones KL, Chatterton BE, Horowitz M.** Scintigraphic measurement of gastric


28. **Perko MJ, Nielsen HB, Skak C, Clemmesen JO, Schroeder TV, Secher NH.** Mesenteric,


35. Schubert MM, Sabapathy S, Leveritt M, Desbrow B. Acute exercise and hormones


41. **ter Steege RW, Van der Palen J, Kolkman JJ.** Prevalence of gastrointestinal complaints in runners competing in a long-distance run: an internet-based observational study in


47. **Victor RG, Leimbach WN Jr, Seals DR, Wallin BG, Mark AL.** Effects of the cold pressor


Figure legends

Figure 1. Schematic representation of study protocol. Subjects ingested the carbohydrate–protein drink either at 5 min (PE-5) or 30 min (PE-30) after high-intensity intermittent cycling exercise and then rested for 60 min. In the control trial (Con), subjects ingested the same nutrient drink without engaging in exercise.

Figure 2. Heart rate responses. The upper, middle, and lower panels indicate nonexercise (Con), 5 min post-exercise (PE-5), and 30 min post-exercise (PE-30), respectively. At 0 min, the vertical dotted line denotes the timing of carbohydrate-protein supplementation.

*: vs. Baseline. +: PE-5 vs. Con. †: PE-5 vs. PE-30. #: Con vs. PE-30. p < 0.05.

Figure 3. Plasma intestinal-fatty acid-binding protein (I-FABP) responses. The upper and lower panels indicate 5 min post-exercise (PE-5) and 30 min post-exercise (PE-30), respectively. At 0 min, the vertical dotted line denotes the timing of carbohydrate–protein supplementation.

*: vs. Baseline. p < 0.05.

Figure 4. Blood flow responses in celiac artery (CA) and superior mesenteric artery (SMA). The upper, middle, and lower panels indicate nonexercise (Con), 5 min post-exercise
(PE-5), and 30 min post-exercise (PE-30), respectively. At 0 min, the vertical dotted line denotes the timing of carbohydrate–protein supplementation. * and (†): vs. baseline, p < 0.05 and p = 0.05, respectively. †: vs. PE-5. p < 0.05.

Figure 5. Relative change in cross-sectional area (CSA) in gastric antrum (i.e., % gastric emptying) following ingestion of drink. Immediately (5 min) after carbohydrate–protein supplementation, CSA of the gastric antrum was defined as 100%. +: Con vs. PE-5. †: PE-30 vs. PE-5. p < 0.05.