

Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise

Shin-ya Ueda, Takahiro Yoshikawa, Yoshihiro Katsura, Tatsuya Usui and Shigeo Fujimoto

Department of Sports Medicine, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

(Correspondence should be addressed to T Yoshikawa; Email: tkhr6719@med.osaka-cu.ac.jp)

Abstract

There is growing interest in the effects of exercise on plasma gut hormone levels and subsequent energy intake (EI) but the effects of mode and exercise intensity on anorectic hormone profiles on subsequent EI remain to be elucidated. We aimed to investigate whether circulating peptide YY₃₋₃₆ (PYY₃₋₃₆) and glucagon-like peptide-1 (GLP-1 or GCG as listed in the HUGO Database) levels depend on exercise intensity, which could affect subsequent EI. Ten young male subjects (mean \pm s.d., age: 23.4 ± 4.3 years, body mass index: 22.5 ± 1.0 kg/m², and maximum oxygen uptake (VO_{2 max}): 45.9 ± 8.5 ml/kg per min) received a standardized breakfast, which was followed by constant cycling exercise at 75% VO_{2 max} (high intensity session), 50% VO_{2 max} (moderate intensity session), or rest (resting session) for 30 min. At lunch, a test meal was presented, and EI was calculated. Blood samples

were obtained during three sessions for measurements of glucose, insulin, PYY₃₋₃₆, and GLP-1, which includes GLP-1 (7-36) amide and GLP-1 (9-36) amide. Increases in blood PYY₃₋₃₆ levels were dependent on the exercise intensity (effect of session: $P < 0.001$ by two-way ANOVA), whereas those in GLP-1 levels were similar between two different exercise sessions. Of note, increase in area under the curve values for GLP-1 levels was negatively correlated with decrease in the EI in each exercise session (high: $P < 0.001$, moderate: $P = 0.002$). The present findings raise the possibility that each gut hormone exhibits its specific blood kinetics in response to two different intensities of exercise stimuli and might play differential roles in regulation of EI after exercise.

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Introduction

Appetite and eating behavior are controlled by a variety of peripheral signals that change in response to food intake and act in the hypothalamus and brainstem (Bray 2000, De Graaf *et al.* 2004). These feedback signals include a number of gastrointestinal hormones, such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1 or GCG as listed in the HUGO Database; Huda *et al.* 2006, Näslund & Hellström 2007, Wren & Bloom 2007). PYY is recognized as a satiety factor (Batterham *et al.* 2002, 2003, Batterham & Bloom 2003), and its precursor peptide, PYY₁₋₃₆, is postprandially released into the circulation from L-cells of the distal ileum and colon. PYY₁₋₃₆ is rapidly metabolized by the enzyme dipeptidyl peptidase-4 (DPP-4), which convert to PYY₃₋₃₆ (Grandt *et al.* 1994a,b). Both these types of PYY (total PYY) exist in human blood, and the PYY₃₋₃₆ is more abundant and more potent in suppressing hunger than PYY₁₋₃₆ (Chelikani *et al.* 2004). GLP-1 is also released into the circulation after a meal in proportion to the amount of food consumed (Kreymann *et al.* 1987), and the major source of the

postprandial GLP-1 release are the L-cells of the intestine (Kieffer & Habener 1999, Gardiner *et al.* 2008). GLP-1 family contains GLP-1 (7-36) amide and GLP-1 (9-36) amide. Besides its action as incretin, GLP-1 inhibits food intake in healthy individuals, diabetics, and nondiabetic obese men (Wynne *et al.* 2005, Druce & Bloom 2006).

Recently, there is growing interest in the effects of exercise on changes in plasma levels of these hormones in healthy subjects (Martins *et al.* 2007, Broom *et al.* 2009). The findings suggest the intriguing possibility that exercise may partly function as a physiological regulator for hormone release or metabolism, and thus yield appetite control. We also demonstrated that a single bout of aerobic exercise caused significant increase in the plasma levels of PYY and GLP-1, and decrease in subsequent energy intake (EI) in obese and nonobese subjects (Ueda *et al.* 2009). Similar to aerobic exercise, vigorous exercise has also been found to significantly reduce hunger (referred to as 'exercise-induced anorexia' (King *et al.* 1994)). It is therefore likely that changes in gut hormones and appetite might vary among different modes and intensities of exercise. While exercise

intensity-dependent changes in plasma levels of orexigenic ghrelin were previously investigated (Erdmann *et al.* 2007), difference in the effects on anorectic gut hormone profiles and subsequent EI between moderate and high intensity of exercise remain to be elucidated.

In the present study, we focused on the two anorectic hormones PYY₃₋₃₆ and GLP-1 commonly produced in enteric L cells and aimed to investigate whether the circulating levels depend on exercise intensity (moderate or high intensity exercise), and thereby affect subsequent EI.

Materials and Methods

Subjects

Ten young male subjects (mean \pm S.D. age: 23.4 ± 4.3 years, body mass index: 22.5 ± 1.0 kg/m², and maximum oxygen uptake (VO_{2 max}): 45.9 ± 8.5 ml/kg per min) were recruited using the student health records of Osaka City University. All subjects were lifelong nonsmokers with a sedentary to moderately active lifestyle (<1 h of intense exercise per day), and reported stable weight and lack of any special type of diet for the previous 6 months. None had any history of infectious disease for at least 1-month period preceding the study, and none were taking medications. Subjects with a history of gastrointestinal, endocrine, cardiovascular, or psychological disease or type 1 or type 2 diabetes were excluded. All subjects provided written informed consent for participation in the study, which was approved by the Ethics Committee of Osaka City University.

Experimental protocol

Prior to the experimental sessions, subjects performed a recumbent ergometer (Strengthgo, Mitsubishi, Tokyo, Japan) ramp exercise test (20 W/min) to determine VO_{2 max} after 3 min rest on the ergometer and a 3 min 0 W warm-up as previously described (Hara *et al.* 2005, Ueda *et al.* 2009). VO_{2 max} was measured with an AE-280S Aeromonitor (Minato Medical Science Inc., Tokyo, Japan). Ventilatory and O₂ consumption variables were calculated using the breath-by-breath method.

All subjects took part in three experimental sessions (high intensity session: 75% VO_{2 max}, moderate intensity session: 50% VO_{2 max}, and resting session) at least 7 days apart. The order of the three sessions was randomized across subjects. To control the subject's physical activity on the days prior to and on the mornings of the experiment of trials, subjects were instructed to refrain from moderate to heavy exercise for at least 24 h prior to each investigation. In addition, all subjects were instructed to consume a weight-maintaining diet containing 55–65% carbohydrate, 10–20% protein, and 20–30% fat (energy percent) at 1 week prior to and throughout the study period. The design of the experimental session is shown diagrammatically in Fig. 1. Subjects received a standard evening meal (instant noodles and a piece of cheese: 532 kcal, 13.9% protein, 26.6% fat, and 59.5% carbohydrate) at around 2100 h on the day preceding each study day. Subjects came to the laboratory at 0930 h and, after a 10-min rest period, a cannula was inserted into an antecubital vein and a fasting venous blood sample (baseline) was taken (20 ml). Then, a standard breakfast (biscuits,

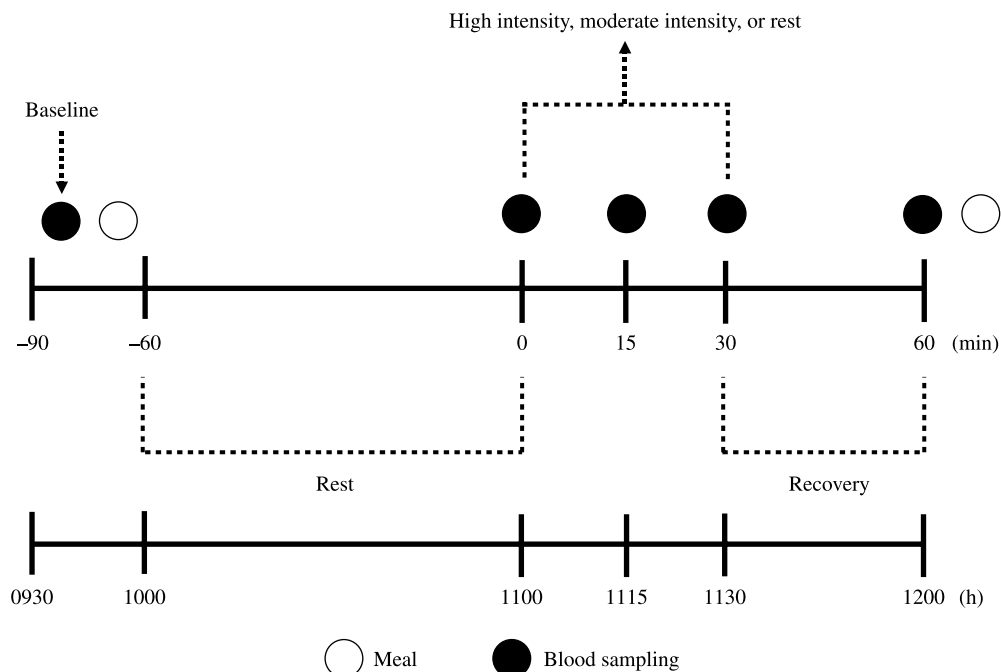


Figure 1 Scheme of the present study.

yogurt, and jelly: 560 kcal, 16.5% protein, 19.3% fat, and 64.2% carbohydrate) was served at 0950 h, and the participants remained seated quietly. At 1100 h ($t=0$ min), the subject either exercised on the recumbent ergometer for 30 min (exercise sessions; moderate or high intensity exercise) or sat, while allowed to read or write quietly (resting session). During these sessions and after the end of the exercise or resting intervention ($t=0, 15, 30, 60$ min), blood samples were obtained. In addition, ratings of subjective feelings of hunger, fullness, satiety, and motivation to eat were reported on a 100 mm visual analogue scale during the study period ($t=0, 15, 30, 60$ min; Flint *et al.* 2000). At 1200 h ($t=60$ min), a test meal (instant pasta: 7.9% protein, 44.6% fat, and 47.5% carbohydrate (energy percent)) was provided, and subjects were instructed to eat as much as they liked until satisfied. In order to exclude the possibility that the amount of food eaten depended on its palatability, we asked all of the subjects which foods they like prior to the study, and selected instant pasta as the test meal. We filled a small bowl with the test pasta and repeatedly filled the bowl with pasta before the participant had emptied it to ensure blindness to the amount of food eaten. No time limit was set for eating under either experimental condition. During the sessions, the subjects and experimenters were instructed to abstain from talking about the meal. Participants were not overtly informed that the true purpose of the present study was to assess feeding responses where possible until they had completed the protocol. After consumption of the test meal, any remaining food was weighed, and the amount determined was subtracted from the premeal value to obtain the total amount of food ingested. Then, absolute EIs from the test meal in each session were calculated from the amount of food eaten (1.15 kcal/g).

Blood sampling

Blood samples were immediately transferred into disodium EDTA-treated tubes for measurement of plasma glucose and hormones. The test tubes were then centrifuged (KUBOTA 2010; KUBOTA Corporation, Tokyo, Japan) at 1400 *g* for 10 min at 4 °C immediately after collection, and the plasma samples were stored at -80 °C until hormone assays. PYY₃₋₃₆ level was determined by EIA (PYY₃₋₃₆ human EIA kit, Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). The inter- and intraassay coefficients of variation (CV) for PYY₃₋₃₆ were 14 and 5% respectively. The assay specifically detects human PYY₃₋₃₆ and has no detectable cross-reactivity with human PYY₁₋₃₆. The cross-reactivity of the antiserum was 100% for human PYY₃₋₃₆. GLP-1 levels including GLP-1 (7-36) amide and GLP-1 (9-36) amide were determined by EIA (GLP-1 EIA kit, Yanaiharu Institute Inc., Shizuoka, Japan). The primary antibody used has 100% cross-reactivity with both GLP-1 (7-36) amide and GLP-1 (9-36) amide, and <1% with other GLP-1 molecules, such as GLP-1 (1-36) amide. The inter- and intraassay CV for GLP-1 were 17 and

10% respectively. Glucose was measured using the enzymatic reference method with hexokinase. Insulin was determined by the chemiluminescence immunoassay (CLIA). All sample measurements were performed in duplicate according to the manufacturer's instructions. Hematocrit (Hct) was measured using a Celltac alpha (Nihon Kohden Inc., Tokyo, Japan).

Statistical analyses

All statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). All data were normally distributed, assessed by the Kolmogorov-Smirnov test, and presented as means \pm S.D. To examine the effects of exercise on sensations of hunger/satiety and on levels of Hct, metabolites and gut hormones, two-way ANOVA with repeated measures was performed. If statistical significance was detected, *post-hoc* multiple pair-wise comparisons (Tukey-Kramer test) were performed.

Areas under the curve (AUC) were calculated using the trapezoidal rule to assess total changes in each gut hormone during each moderate and high intensity exercise ($t=0-60$; AUC_{PYY} (Moderate), AUC_{PYY} (High), AUC_{GLP-1} (Moderate), and AUC_{GLP-1} (High)), and during the resting session ($t=0-60$; AUC_{PYY} (Rest) and AUC_{GLP-1} (Rest)). Total increase in gut hormone (PYY₃₋₃₆ and GLP-1) levels during each moderate and high intensity session from the levels at resting session was calculated as follows:

$$\Delta\text{AUC}_{\text{PYY}}(\text{Moderate})$$

$$= \text{AUC}_{\text{PYY}}(\text{Moderate}) - \text{AUC}_{\text{PYY}}(\text{Rest}),$$

$$\Delta\text{AUC}_{\text{PYY}}(\text{High}) = \text{AUC}_{\text{PYY}}(\text{High}) - \text{AUC}_{\text{PYY}}(\text{Rest}),$$

$$\Delta\text{AUC}_{\text{GLP-1}}(\text{Moderate})$$

$$= \text{AUC}_{\text{GLP-1}}(\text{Moderate}) - \text{AUC}_{\text{GLP-1}}(\text{Rest}),$$

$$\Delta\text{AUC}_{\text{GLP-1}}(\text{High}) = \text{AUC}_{\text{GLP-1}}(\text{High}) - \text{AUC}_{\text{GLP-1}}(\text{Rest}).$$

Also, decrease in amount of energy ingested following each moderate and high intensity session (EI (Moderate) and EI (High)) compared with that after resting session (EI (Rest)) was estimated as follows:

$$\Delta\text{EI}(\text{Moderate}) = \text{EI}(\text{Moderate}) - \text{EI}(\text{Rest}),$$

$$\Delta\text{EI}(\text{High}) = \text{EI}(\text{High}) - \text{EI}(\text{Rest}),$$

To determine the impact of session on EI and AUC, one-way ANOVA with repeated measures was performed. If statistical significance was detected, *post-hoc* multiple pair-wise comparisons (Tukey-Kramer test) were performed.

In addition, correlation between Δ AUC and Δ EI was determined by simple correlation Pearson's correlation coefficients. P values <0.05 were considered significant.

Results

Blood parameters

Figure 2 shows changes over time in blood parameters. None of the baseline values of the measured variables differed significantly among sessions. PYY₃₋₃₆ and GLP-1 levels were significantly increased during 30 min exercise (PYY₃₋₃₆; main effect of session: $P<0.001$, main effect of time: $P<0.001$, and session \times time interaction effect: $P<0.001$, GLP-1; main effect of session: $P<0.001$, main effect of time: $P<0.001$, and session \times time interaction effect: $P<0.001$). The increase in PYY₃₋₃₆ levels was maintained after the high intensity exercise than after the moderate exercise (*post-hoc* test: $P=0.020$, at $t=60$ min), and the mean AUC values observed for PYY₃₋₃₆ depend on exercise intensity (Table 1). In contrast, the post-exercise increases in the GLP-1 levels were maintained after both high and moderate intensity

exercise, and there were no significant differences in the mean AUC values for GLP-1 between two exercise sessions (Table 1). Glucose levels were suppressed during exercise (main effect of session: $P=0.037$, main effect of time: $P<0.001$, and session \times time interaction effect: $P<0.001$). No significant main effect of session was observed for insulin level ($P=0.385$).

Changes in Hct over the period of observation were small and did not differ among three sessions (main effect of session: $P=0.361$). The study results were unlikely to be affected by hemoconcentration during the exercise sessions in the present study.

Absolute EI and measures of appetite

A significant main effect of session was observed for amount of EI at noon ($P<0.001$; Fig. 3). EI was significantly lower after the high intensity and moderate intensity exercise sessions compared with that after the resting session (*post-hoc* test; $P<0.01$ respectively). However, the mean values of EI in the high intensity session were not significantly different from those in moderate intensity exercise session. Hunger scores were significantly suppressed during and after exercise

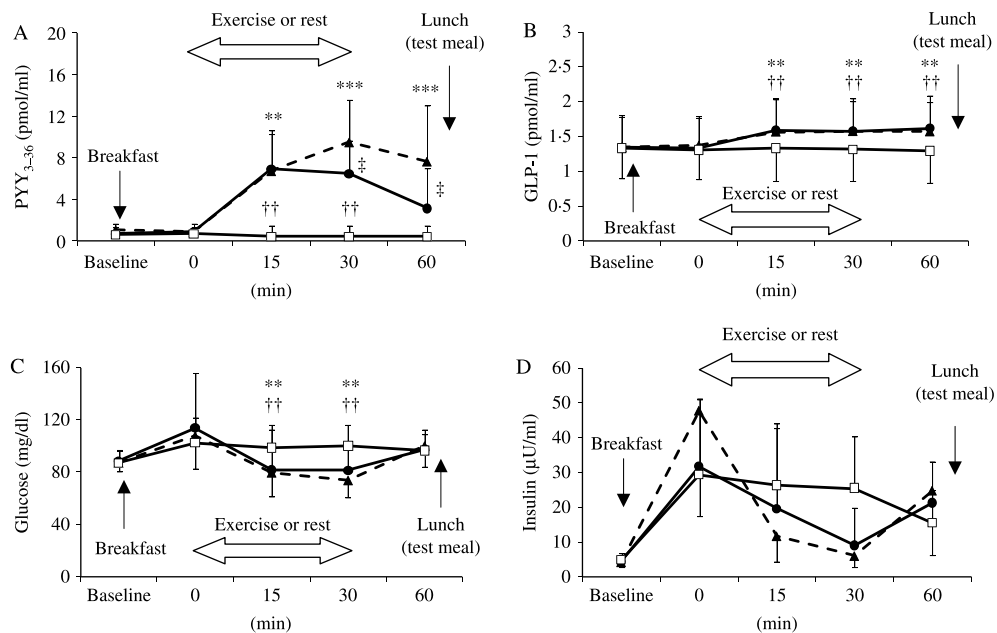


Figure 2 Time course of changes in plasma levels of PYY₃₋₃₆ (A), GLP-1 (B), glucose (C), and insulin (D) during high intensity (▲), moderate intensity (●), and resting (□) sessions. Mean values \pm s.d. of each parameter are presented. Two-way ANOVA for repeated measures: (A) main effect of session, $P<0.001$; main effect of time, $P<0.001$; interaction effect of session \times time, $P<0.001$. ** $P<0.01$ and *** $P<0.001$, resting versus high intensity sessions. †† $P<0.01$, resting versus moderate intensity sessions. † $P<0.05$, moderate intensity versus high intensity sessions. (B) Main effect of session, $P<0.001$; main effect of time, $P<0.001$; interaction effect of session \times time, $P<0.001$. ** $P<0.01$, resting versus high intensity sessions. †† $P<0.01$, resting versus moderate intensity sessions. (C) Main effect of session, $P=0.037$; main effect of time, $P<0.001$; interaction effect of session \times time, $P<0.001$. ** $P<0.01$, resting versus high intensity sessions. †† $P<0.01$, resting versus moderate intensity sessions. (D) Main effect of session: $P=0.385$, main effect of time: $P<0.001$, and interaction effect of session \times time: $P<0.001$.

Table 1 Area under the curve values for peptide YY₃₋₃₆ (PYY₃₋₃₆) and glucagon-like peptide-1 (GLP-1). All values are described as mean \pm s.d. Findings were analyzed using one-way ANOVA and Tukey–Kramer *post-hoc* tests.

	High	Moderate	Resting	Significance
PYY ₃₋₃₆ (pmol/ml \times 60 min)	446.8 \pm 187.0	293.4 \pm 171.9	26.7 \pm 16.2	*,* [§]
GLP-1 (pmol/ml \times 60 min)	94.8 \pm 27.0	95.3 \pm 28.7	80.3 \pm 28.4	†,‡

* $P < 0.001$ and † $P < 0.01$, resting versus high intensity exercise session. ‡ $P < 0.01$, resting versus moderate intensity exercise session. § $P < 0.05$, high intensity exercise versus moderate intensity exercise session.

(main effect of session: $P = 0.021$, main effect of time: $P < 0.001$, and session \times time interaction effect: $P = 0.045$; Fig. 4). However, no significant changes were observed in fullness, satiety, or motivation to eat in response to exercise (data not shown).

Correlations of increase in hormone levels and decrease in EI

Significant negative correlations were observed between the total increase in GLP-1 levels during each exercise session from the levels at resting session and the decrease in the amount of energy ingested ($\Delta\text{AUC}_{\text{GLP-1}}$ (High) versus ΔEI (High): $r = -0.893$, $P < 0.001$; $\Delta\text{AUC}_{\text{GLP-1}}$ (Moderate) versus ΔEI (Moderate): $r = -0.816$, $P = 0.002$; Fig. 5). In contrast, no significant correlations between the delta of AUC values for PYY₃₋₃₆ concentrations and change in the amount of energy ingested were observed (data not shown).

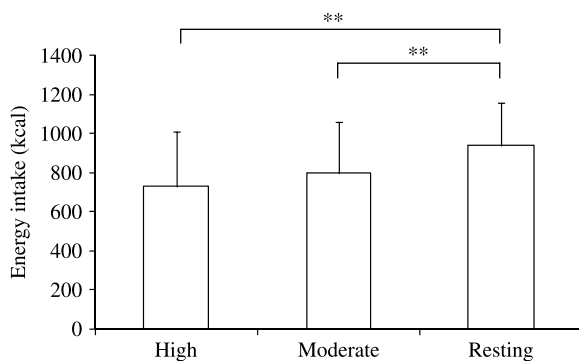
Discussion

The objective of the present study was to determine whether circulating PYY₃₋₃₆ levels and GLP-1 levels depend on exercise intensity (moderate or high intensity), and thereby affect subsequent EI. The following findings were obtained: 1) increases in blood PYY₃₋₃₆ levels were dependent on the intensity of exercise, whereas those in GLP-1 levels were similar between two different intensities, 2) significant reduction in amount of energy ingested was observed in both moderate and high intensity of exercise sessions

compared with resting session, but the decrease was not significantly different between two intensities of exercise, 3) increase in AUC values for GLP-1 levels was significantly and negatively correlated with decrease in the amount of energy ingested in each intensity session.

Findings of an increasing number of studies, so far, have indicated that the circulating level of total PYY could be affected by a single bout of exercise (Martins *et al.* 2007, Broom *et al.* 2009, Ueda *et al.* 2009). Among the members of the PYY peptide family, PYY₃₋₃₆ in particular, has been shown to play a major role in appetite control (Chelikani *et al.* 2004). To the best of our knowledge, this is the first study to date that has examined the effects of a single bout of exercise on the blood level of PYY₃₋₃₆. We demonstrated that acute exercise transiently increases blood PYY₃₋₃₆ levels, similar to the time course of changes in blood total PYY level observed in previous studies (Martins *et al.* 2007, Broom *et al.* 2009, Ueda *et al.* 2009). Interestingly, we found that higher intensity of exercise caused greater increase in blood PYY₃₋₃₆ levels above resting session levels compared with moderate intensity sessions, suggesting that increase in PYY₃₋₃₆ levels depends on exercise intensity.

Similar to the present study, a transient increase in the plasma levels of GLP-1 in response to acute exercise was previously shown in athletes (O'Connor *et al.* 1995, 2006) and in healthy unrestrained, normal weight volunteers (Martins *et al.* 2007). The present finding first demonstrated that, while PYY₃₋₃₆ levels were more strongly increased in high intensity sessions than in those of moderate intensity, no significant difference was observed in GLP-1 levels between these two sessions. In other words, moderate exercise can partially activate the PYY secretion, and high intensity exercise can further enhance the secretion activity, while moderate intensity exercise can stimulate GLP-1 secretion comparable in magnitude to high intensity exercise. So far, *in vitro* study showed that PYY and GLP-1 are colocalized in endocrine L-cells, and both are synchronically secreted from these cells of rat isolated distal ileum after direct administration of intestinal regulatory peptides (Dumoulin *et al.* 1995). In human postprandial study, a concurrent increase in human plasma PYY and GLP-1 concentrations was observed after a meal, but the increment and duration of each hormone depended on the size and nature (nutrients) of the meal given (Adrian *et al.* 1985, Elliott *et al.* 1993), indicating that secretion kinetics could be driven by the type and intensity of nutrient stimuli to secretory cells. Similarly, the finding of

**Figure 3** Absolute amount of energy ingested in the test meal during three sessions. All values are means \pm s.d. ** $P < 0.01$.

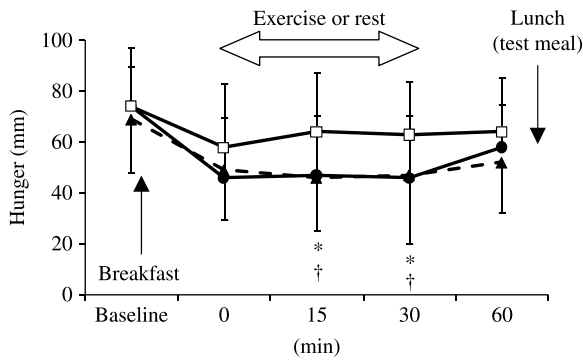


Figure 4 Time course of changes of hunger scores during high intensity (▲), moderate intensity (●), and resting (□) sessions. Mean values \pm s.d. of each parameter are presented. Two-way ANOVA for repeated measures: main effect of session, $P=0.021$; main effect of time, $P<0.001$; interaction effect of session \times time, $P=0.045$. * $P<0.05$, resting versus high intensity sessions. † $P<0.05$, resting versus moderate intensity sessions.

the present study raises the possibility that kinetics of each hormone in blood might differ among different types and intensities of exercise as well as nutrient stimuli. The release of these hormones is known to be under both neural and endocrine influence as well as being stimulated directly by luminal nutrients (Huda *et al.* 2006). Another point of note is that, in addition to the common release sites, these hormones share the metabolizing system through the enzyme DPP-4. PYY₁₋₃₆ is rapidly metabolized by DPP-4 converting to PYY₃₋₃₆ (Medeiros & Turner 1994), whereas GLP-1 (7-36) amide is subject to rapid breakdown by the actions of DPP-4, generating GLP-1 (9-36) amide (Deacon *et al.* 1995). Thus, it seems worthwhile to investigate the involvement of this enzyme in the blood kinetics of these gut hormones during and after exercise for interpreting the present findings. Collectively, the present finding may provide new insights into the mechanisms of change in PYY₃₋₃₆ and GLP-1 by exercise in humans.

Another finding is that the amount of energy ingested was significantly reduced after moderate intensity exercise compared with that of resting session and the decrease in amount was comparable with that after high intensity in the present study setting (Fig. 3). There are mixed findings on the acute effects of various intensities of exercise on subsequent macronutrient intake (Elder & Roberts 2007), and such conflicting results may partly stem from subject variables (sex, body fat level, and exercise history) and study setting (exercise duration, measure of the exercise intensity (Watt or $\text{VO}_2 \text{max}$), time interval between exercise and food intake, and meal style). Of particular interest in the present study is that, in contrast to PYY₃₋₃₆, the reduction in amount of energy ingested (ΔEI (Moderate) and ΔEI (High)) was significantly and negatively associated with the total increase in the GLP-1 concentrations during moderate intensity exercise ($\Delta\text{AUC}_{\text{GLP-1}}$ (Moderate)) and high intensity exercise ($\Delta\text{AUC}_{\text{GLP-1}}$ (High)) respectively (Fig. 5). In addition, an

inverse pattern of time course curve was observed between hunger and two hormone levels, consistent with the previous study (Martins *et al.* 2007). Interestingly, there were no significant differences between moderate and high intensity sessions in the curve of hunger and that of GLP-1 levels (Figs 2 and 4). One of the possible interpretations for these findings is that, in this study setting, GLP-1 is more closely involved in exercise-induced suppression of hunger and amount of subsequent EI after moderate and high intensity exercise compared with PYY₃₋₃₆. Both PYY and GLP-1 are known to act peripherally as an 'ileal brake', slowing nutrient transit, ensuring more complete nutrient absorption in the small intestine, and terminating feeding by inducing satiety effects on the brain. In addition, both of these hormones can directly communicate with the hypothalamic and brainstem circuits that control appetite and energy expenditure by blood-borne mechanisms (Huda *et al.* 2006). Although we cannot clearly distinguish between the effects of PYY and GLP-1 endogenously induced by exercise stimuli on appetite control in this experiment design alone, it is likely that these anorectic hormones might play differential roles for appetite regulatory processes after exercise. Furthermore, simultaneous measurements of gastric emptying rate and circulating levels of each gut hormone during and after exercise sessions seem to yield valuable information for interpreting the present findings more accurately.

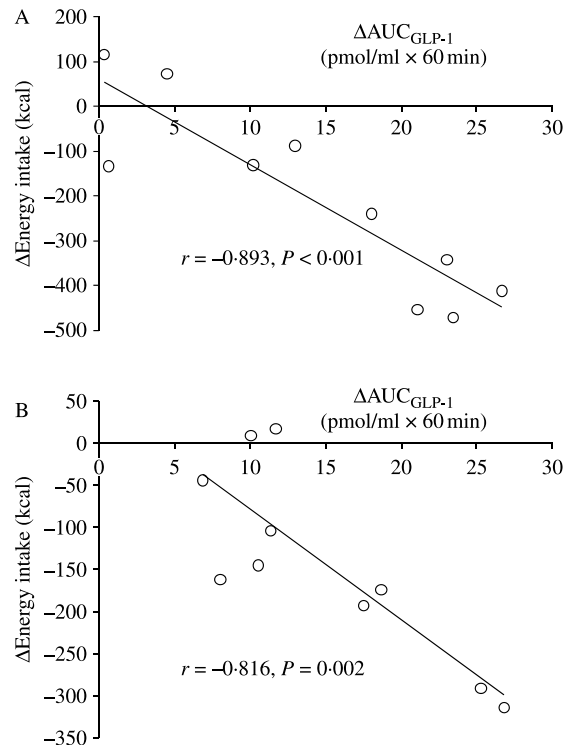


Figure 5 Correlation between incremental GLP-1 response and the decrease in the amount of energy ingested in high intensity exercise session (A) and in moderate intensity session (B).

There are some potential limitations to the present study. First, we did not calculate the energy expenditure during the whole time course from breakfast until lunch time in each session, but simply expressed as amount of energy ingested at lunch. In the present studies, we focused on how much energy subjects can consume at lunch after each session. In future studies, it is also worthwhile to evaluate the energy balance in the long observation following each session rather than to assess a short-term energy balance only during the morning on each test day. Additionally, to identify a true exercise intensity effect, it will be necessary to match the energy expenditure between two intensity sessions by halting the exercise once a specified energy expenditure goal has been reached. Therefore, we should calculate by frequent expired air measurements throughout the experiment. Secondly, we recruited a small number of subjects in the present study, and we could not estimate exact statistical power. Despite the small sample size, the present findings have raised the possibility that circulating PYY₃₋₃₆ levels and GLP-1 levels were differentially associated with exercise intensity (moderate intensity exercise or high intensity exercise), and thereby affect subsequent EI. Study of a large population will be needed to confirm the present findings. Thirdly, we aimed to highlight two anorectic gut hormones, PYY and GLP-1, in the present study because enteric L cells are common major sources of these two gut hormones. However, it is obvious that, beyond these hormones, various physiological (other appetite-related hormones), psychological (cognitive factor, motivation to eat, dietary restraint, and palatability), and other factors (gender, body weight) collectively determine overall appetite and amount of EI after acute exercise (King 1999, Martins *et al.* 2008). Among many physiological factors, a previous study (Erdmann *et al.* 2007) reported that orexigenic ghrelin concentrations significantly rose in the fasting state by exercise on ergometer for 30 min at 50 W, which was lower intensity than aerobic exercise but the hormone levels remained unchanged during the high intensity at 100 W, and that the hunger and satiety ratings were not significantly different between two intensity sessions, suggesting that low rather than high intensity exercise stimulates ghrelin levels, and the changes in the ghrelin was not directly associated with appetite in the study setting. In the present study, subjects were instructed to eat the standard breakfast before each exercise session. It will be necessary to investigate the effects of exercise intensity on changes in gut hormones and subsequent EI in a common study protocol. We designed to minimize the confounding effects, such as gender, body weight, cognitive factor, and palatability, in a manner similar to the study previously reported (Ueda *et al.* 2009). Regarding body weight, we previously reported that decrease in EI by a single bout of aerobic exercise was significantly larger in obese subjects compared with nonobese subjects despite similarity in total amount of PYY and GLP-1 secretions between groups, suggesting that EI and energy balance during single bout of aerobic exercise might be influenced not only by the anorectic gut hormones but also by other factors such as

obesity (Ueda *et al.* 2009). Thus, it also seems necessary to examine whether the findings observed in the present study can also be reproduced in obese subjects.

In summary, circulating levels of PYY₃₋₃₆ rose with increase in exercise intensity but are poorly associated with subsequent EI, whereas the blood kinetics of GLP-1 levels in moderate intensity exercise are comparable with that in high intensity exercise, and the increment of the plasma levels of GLP-1 proportionally suppress the subsequent EI after both types of exercise. The present findings raise the possibility that each gut hormone exhibits its specific blood kinetics in response to different intensities of exercise stimuli and might differentially regulate amount of EI after exercise. Further elucidation of physiological characteristics and roles of each gut hormone on EI in response to various types of exercise stimuli will likely help to develop exercise programs more appropriate to individual nutritional needs in athletes or obese subjects. Our findings deserve further evaluation in studies involving other types of exercise (e.g. incremental exercise) and subjects with other characteristics (e.g. obese subjects).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S-y U participated in the acquisition of data, analysis and interpretation of data, and drafting of the manuscript, and approved the final version. He received 10 000\$ in 2009 from the Meiji-Yasuda Life Foundation of Health and Welfare. T Y participated in the critical conception and design, interpretation of data critical revision of the manuscript, and approved the final version. He has no conflict of interest. Y K participated in the acquisition of data and approved the final version. He has no conflict of interest. T U participated in the acquisition of data and approved the final version. He has no conflict of interest. S F participated in acquisition of data and approved the final version. He also has no conflict of interest.

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