Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males

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

Abstract

We examined whether changes in gut hormone levels due to a single bout of aerobic exercise differ between obese young males and normal controls, and attempted to determine the involvement of hormonal changes during exercise in the regulation of energy balance (EB) in these obese subjects. Seven obese and seven age-matched subjects of normal weight participated in exercise and rest sessions. Subjects consumed a standardized breakfast that was followed by constant cycling exercise at 50% VO2max or rest for 60 min. At lunch, a test meal was presented, and energy intake (EI) and relative energy intake (REI) were calculated. Blood samples were obtained at 30 min intervals during both sessions for measurement of glucose, insulin, glucagon, ghrelin, peptide YY (PYY), and glucagon-like peptide-1 (GLP-1). Plasma levels of PYY and GLP-1 were increased by exercise, whereas plasma ghrelin levels were unaffected by exercise. The areas under the curve (AUC) of the time courses of PYY and GLP-1 levels did not significantly differ between the two groups. In contrast, EI and REI were decreased by exercise in both groups, and energy deficit was significantly larger in obese subjects than in normal controls. The present findings suggest that short-term EB during a single exercise session might be regulated not by increased amounts of these gut hormones per se.

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Introduction

Excess body weight is a major health problem that affects increasing numbers of people worldwide (Strauss & Pollack 2001, Ogden et al. 2002, Flegal 2005). Despite the multifactorial etiology of obesity, it seems plausible that behavioral and environmental changes, such as a modern sedentary lifestyle combined with increased dietary intake, have significantly contributed to the recent rapid increase in the prevalence of obesity. It is therefore believed that lifestyle modification based on healthy diet and increased physical activity is of great importance to health (Bianchi et al. 2008). This approach, however, often compels obese patients to fight cravings for food. It thus appears important to control the appetite of obese patients in addition to changing their amount and patterns of food consumption. A substantial body of research on the association of exercise with food intake has suggested that physical activity has an impact not only on energy expenditure but also on variations in appetite and post-exercise energy intake (EI), leading to negative energy balance (EB; Blundell & King 1998, Hubert et al. 1998).

Recently, there has been growing concern that appetite and energy homeostasis are controlled by a variety of peripheral signals that change in response to starvation or food intake and act in the hypothalamus and brainstem to alter feelings of hunger or fullness so as to determine meal initiation (hunger) or meal termination (satiety; Bray 2000, De Graaf et al. 2004). These feedback signals may include a number of ascending neural inputs (e.g., signaling via the vagal nerve of gastric distension) and hormonal changes, including those of orexigenic or anorexigenic hormones released from gastrointestinal or endocrine organs, including ghrelin, peptide YY (PYY), pancreatic polypeptide (PP), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK), and oxyntomodulin (Huda et al. 2006, Nislund & Hellstrom 2007, Wren & Bloom 2007). While of these, ghrelin is the only orexigenic endogenous hormone, the other peptides and hormones have been found to have anorexigenic effects.

Interestingly, recent studies have revealed inhibitory effects of exercise on the hunger associated with these hormones in healthy subjects (Martins et al. 2007), suggesting the intriguing possibility that exercise may promote a favorable gut hormone profile yielding sustained appetite control and weight loss. Clear understanding of the changes in gut hormones during exercise in obese subjects will aid the development of optimal measures for the prevention and treatment of this condition. To the best of our knowledge, however, few studies have shed light on the effects of exercise on EB and weight reduction through exercise-induced hormonal activities. In addition, it is unknown whether gut hormone release during exercise differs between subjects who are obese and those with normal body weight.
In the present study, we examined whether changes in plasma gut hormone levels induced by a single bout of aerobic exercise of moderate intensity for 1 h differed between obese young males and age-matched controls of normal body weight, and determined the involvement of these gut hormones in the regulation of EB in each subject group.

Materials and Methods

Subjects

Fourteen young male subjects (seven obese subjects and seven control subjects with normal body weight) were recruited using the student health records of Osaka City University. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. In accordance with the definition of the Japan Society for the Study of Obesity (Hara et al. 2005, Kondo et al. 2006), we considered participants obese if their BMI was $\geq 25$ kg/m$^2$ and normal in weight if their BMI was $<25$ kg/m$^2$ (BMI range; normal controls: 19.1–24.7, obese subjects: 26.0–34.6). All subjects were lifelong non-smokers with a sedentary to moderately active lifestyle (less than one hour 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 the 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. The characteristics of the subjects are shown in Table 1. All subjects provided written informed consent for participation in the study, which was approved by the Ethics Committee of Osaka City University.

Exercise performance test

Prior to the two experimental sessions, subjects performed a recumbent ergometer (Strenghero, Mitsubishi, Tokyo, Japan) ramp exercise test (20 W/min) to determine VO$_{2\text{max}}$ after 3 min rest on the ergometer and a 3 min 0 W warm-up. VO$_{2\text{max}}$ was measured with an AE-280S Aeromonitor (Minato Medical Science Inc., Tokyo, Japan). The system consisted of a microcomputer, a hot-wire flow meter, and a gas analyzer, containing a sampling tube, filter, suction pump, and O$_2$ analyzer composed of a zirconium element and an infrared CO$_2$ analyzer. Ventilatory and O$_2$ consumption variables were calculated using the breath-by-breath method. The electrocardiogram and heart rate (HR) were continuously monitored with a Dyna Scope (DS-3140, Fukuda Denshi, Tokyo, Japan) throughout the ramp exercise test. Perceived exertion was rated every minute using the Borg scale.

Experimental protocol

Subjects took part in two experimental sessions (in exercise and resting conditions) at least 7 days apart. The order of the two sessions was randomized across subjects. 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 g protein, 26.6 g fat, and 59.5 g carbohydrate) at around 21:00 h on the day proceeding each of the study days. Subjects came to the laboratory at 8:30 h and after a 10 min rest period, a cannula was inserted into an antecubital vein and a fasting venous blood sample ($t=-60$ min) was taken (20 ml). Then a standard breakfast (biscuits, yogurt, and jelly: 560 kcal, 18.6 g protein, 21.6 g fat, and 72.3 g carbohydrate) was served at 8:50 h and the participants remained seated quietly. At 10:00 h, ($t=0$ min), the subject either exercised on the recumbent ergometer at 50% VO$_{2\text{max}}$ for 60 min (exercise session) or sat while allowed to read or write quietly (resting session). During these sessions and after the end of the exercise or resting intervention ($t=0$, 30, 60, 90, 120 min), blood samples were obtained at 30 min intervals. In addition, ratings of subjective feelings of hunger and satiety were reported on a 100 mm visual analogue scale during the study period ($t=-60$, 0, 30, 60, 90, 120 min; Flint et al. 2000). At 12:00 noon ($t=120$ min), a test meal (instant pasta: 1.15 kcal/g) 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 the subjects which foods they liked 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 to the extent possible not overtly informed that the true purpose of the present study was to assess feeding responses until they had completed the protocol.

Table 1 Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Body weight (kg)</th>
<th>BMI (kg/m$^2$)</th>
<th>Body fat (%)</th>
<th>Waist (cm)</th>
<th>VO$_{2\text{max}}$ (ml/kg per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>22.4±4.2</td>
<td>170.8±2.9</td>
<td>65.3±7.3</td>
<td>22.4±2.4</td>
<td>15±1±4</td>
<td>74.1±5.5</td>
<td>46.6±3.9</td>
</tr>
<tr>
<td>Obese</td>
<td>22.9±3.4</td>
<td>172.8±4.6</td>
<td>89.7±11.9</td>
<td>30.0±3.1</td>
<td>26±4±3.7</td>
<td>96.6±5.3</td>
<td>34.0±6.3</td>
</tr>
</tbody>
</table>

All values are described as mean±s.d. *P<0.001; normal versus obese subjects.

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Energy intake, energy expenditure, and relative energy intake

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 the exercise session (EI (Ex)) and resting session (EI (R)) were calculated from the amount of food eaten (1.15 kcal/g). Energy expenditures during the exercise (EE (Ex)) and resting sessions (EE (R); t = 0 to 120 min) were estimated based on metabolic equivalents as previously described (Ainsworth et al. 2000). REI from the test meal corresponds to EI corrected for the energy expended during exercise (REI (Ex)) and resting (REI (R)) sessions, and is calculated as follows

\[
\text{REI}(\text{Ex}) = \text{EI}(\text{Ex}) - \text{EE}(\text{Ex}), \quad \text{REI}(\text{R}) = \text{EI}(\text{R}) - \text{EE}(\text{R}).
\]

Hormone measurements

Blood samples were immediately transferred into disodium EDTA-treated tubes for measurement of plasma glucose and hormones. In addition, aprotinin, a potent protease inhibitor, was added to the samples at a concentration of 500 kIU/ml for measurement of ghrelin. The test tubes were then centrifuged at 3000 r.p.m. for 15 min at 4 °C immediately after collection, and the plasma samples were stored at −80 °C until hormone assays. Insulin was determined by the fully automated chemiluminescence method. Glucagon level was determined by RIA. Glucose was measured using the enzymatic reference method with hexokinase. Plasma PYY and GLP-1 (GLP-1 (7–36) amide) levels were determined by EIA (Human PYY/GLP-1 EIA kit, Yanahara Institute Inc., Shizuoka, Japan). The ELISA for PYY quantified the total amount of both PYY\textsubscript{1–36} and PYY\textsubscript{3–36}. Plasma ghrelin levels were assessed by ELISA (Active Ghrelin ELISA kit, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). The interassay coefficients of variation for PYY, GLP-1, and ghrelin were each less than 18%. The sensitivities (minimum limits of detection) of PYY, GLP-1, and ghrelin were 0.03 and 0.062 pmol/ml and 2.5 pmol/l, respectively. All sample measurements were performed in duplicate according to the manufacturers’ instructions.

Statistical analyses

All statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). All data were normally distributed, and presented as means ± S.D. The unpaired t-test was used for comparison of baseline characteristics between obese and normal control males.

To examine the effects of exercise on sensations of hunger/satiety and on levels of hemoglobin, hematocrit, metabolites, and gut hormones, two-way ANOVA with repeated measures was performed in each body-type group. If statistical significance was detected, post-hoc multiple pairwise comparisons (Tukey–Kramer test) were performed.

Areas under the curve (AUCs) were calculated using the trapezoidal rule to assess total hormonal changes during the exercise session (t = 0 to 120; AUC\textsubscript{PYY} (Ex), AUC\textsubscript{GLP-1} (Ex), AUC\textsubscript{ghrelin} (Ex)) and during the resting session (t = 0 to 120; AUC\textsubscript{PYY} (R), AUC\textsubscript{GLP-1} (R), AUC\textsubscript{ghrelin} (R)). In addition, to determine the impact of obesity on EI, REI, and AUC, two-way ANOVA with repeated measures was performed. P values less than 0.05 were considered significant.

Results

EI and REI

Mean values of EI and REI after exercise sessions were significantly lower than those after resting sessions (EI and REI; P < 0.001). In addition, there were significant differences in EI and REI between normal control and obese males (EI, P = 0.038; REI, P = 0.021), suggesting the presence of a greater energy deficit due to exercise in obese subjects than in normal control subjects (Table 2).
Hemoglobin and hematocrit

No significant changes in either hemoglobin or hematocrit were observed over time during the exercise or resting sessions (Fig. 2). Hemoconcentration was thus unlikely to have occurred during the exercise sessions performed in the present study.

Metabolites, gut hormone concentrations, and measures of appetite

Figure 3 shows the time courses of glucose, insulin, and glucagon levels in blood from \( t = -60 \) to 120. No significant difference in levels of glucose was observed between the exercise and resting sessions throughout the course of observation. Despite a significant change in insulin level after breakfast, these levels were not significantly affected by exercise. On the other hand, plasma glucagon levels were increased during the 1 h exercise period and after the end of exercise compared with those in resting sessions.

The time courses of gut hormones levels are presented in Fig. 4. Following the onset of exercise \( (t = 0 \) to 120), mean levels of PYY and GLP-1 significantly increased in the exercise session compared with those in the resting session as determined by two-way ANOVA for repeated measures (PYY: normal: \( P = 0.011 \), obese: \( P = 0.020 \); GLP-1: normal: \( P = 0.041 \), obese: \( P = 0.002 \)), while plasma ghrelin levels during exercise did not differ from those in the resting session. In addition, during the recovery period \( (t = 60 \) to 120), mean PYY levels fell to those in the resting session, while the increase in level of GLP-1 was maintained compared with the resting session (Fig. 4). No significant changes were observed in hunger, fullness, satiety or motivation to eat in response to exercise in either the normal or obese males (data not shown).

In both of the subject groups, mean AUC values for PYY and GLP-1 were higher in the exercise session than those observed in the resting session (effect of sessions; PYY;

<table>
<thead>
<tr>
<th>Normal control (n=7)</th>
<th>Obese (n=7)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting (R)</td>
<td>Exercise (Ex)</td>
<td>Resting (R)</td>
</tr>
<tr>
<td>El (kcal)</td>
<td>838.2 ± 113.6</td>
<td>692.3 ± 106.9</td>
</tr>
<tr>
<td>REI (kcal)</td>
<td>632.4 ± 116.4</td>
<td>196.3 ± 108.1</td>
</tr>
</tbody>
</table>

All values are described as mean ± s.d. ANOVA for repeated measures. *\( P < 0.001 \); effect of session (resting or exercise). †\( P < 0.05 \); effect of subject group (normal control or obese males).
However, the mean values of AUC_PYY and AUC/GLP-1 in obese subjects were not significantly different from those in normal control subjects (effect of subject groups; PYY; P > 0.121, GLP-1; P > 0.168; Table 3). These findings suggest that, despite the larger energy deficit in obese subjects than in normal control subjects (Table 2), the increases in the levels of anorexigenic hormones PYY and GLP-1 by exercise were not significantly different between these subject groups.

**Discussion**

The objectives of this study were to compare changes in the release of gastrointestinal hormones after a single bout of aerobic exercise in young obese subjects with those in normal-weight subjects, and to compare physiological involvement of these hormones in regulation of food intake and EB after exercise in obese subjects with that in normal controls. The following findings were obtained: (1)
In both groups, 1 h cycling exercise (50% VO$_{2\text{max}}$) significantly increased subsequent release of PYY and GLP-1 in plasma, while plasma levels of ghrelin were not significantly altered. (2) Exercise-induced suppression of EI in obese subjects was significantly larger than in control subjects despite the lack of significant difference in PYY or GLP-1 levels between groups.

There is considerable evidence that PYY, GLP-1, and ghrelin play roles in short-term regulation of appetite and energy homeostasis both in subjects of normal weight...
VO2max of each subject was measured to ensure that intensity
hormonal condition) affected findings, female subjects were
subjects. To exclude the possibility that gender (e.g.,
plays a role in the regulation of EB in obese as well as normal
single bout of exercise of moderate intensity and whether this
release of these anorexigenic gut hormones increases during a
in both types of subjects. We therefore examined, whether
normal subjects, the increase in anorexigenic hormone release
levels of these anorexigenic gut hormones during a single
exercise might affect subsequent EB in subjects with normal
the anorexigenic hormones PYY and GLP-1, but not that of
65% of maximal HR significantly increased plasma levels of
ghrelin may be therapeutically useful in obese overeating
subjects. Obese subjects may be more sensitive to stimulation
concentrations of ghrelin than age-matched lean control
in anorexigenic gut hormones induced by a single bout of exercise did not
significantly differ from those observed at rest, consistent with
previous findings (Kallio et al. 2001, Dall et al. 2002, Kraemer et al. 2004, Schmidt et al. 2008) although
other studies have reported plasma ghrelin levels decrease
with exercise (Toshinai et al. 2007, Malkova et al. 2008) or increased (Erdmann et al. 2007) with exercise. These results
might depend on the setting of exercise and the timing of food intake. Our findings suggest that, after meal intake, the impact
of a single exercise session on regulation of plasma ghrelin level is less than those on other gut hormones in both obese
and normal subjects.

Our study has some limitations. First, we cannot rule out
the possibility that cognitive or environmental factors affected
our findings, although we carefully attempted to exclude
such confounding variables by the choice of study design.
When allowed to eat ad libitum, obese subjects consume more
food items than do subjects with normal weight (Wing et al.
1978). In fact, in previous studies of appetite in obese
subjects, subjects were instructed to eat ad libitum (Dall et al.
2002). However, under these circumstances, amount of food intake can be biased by cognitive factors such as the belief
that ‘food is a reward for exercise’ (King 1999). In the present
study, a common test meal of noodles was therefore prepared
so that subjects would be unaware of the amount of food
take during the test. In addition, prior to the study, we
confirmed that the test meal was palatable to all participants.
Second, the mechanisms underlying the time-course changes in plasma PYY and GLP-1 during exercise remain unclear
in detail. PYY is rapidly released within 15 min after food

Table 3 Gut hormones output during the exercise and resting sessions

<table>
<thead>
<tr>
<th></th>
<th>Normal control (n=7)</th>
<th>Obese (n=7)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting (R)</td>
<td>Exercise (Ex)</td>
<td>Resting (R)</td>
</tr>
<tr>
<td>AUCPYY (pmol/ml×120 min)</td>
<td>213.3±51.2</td>
<td>237.7±54.1</td>
<td>182.3±23.1</td>
</tr>
<tr>
<td>AUCGLP-1 (pmol/ml×120 min)</td>
<td>131.8±77.6</td>
<td>176.8±104.3</td>
<td>96.2±35.4</td>
</tr>
<tr>
<td>AUCghrelin (pmol/l×120 min)</td>
<td>5911.8±1299-2</td>
<td>6864.7±1385.1</td>
<td>9364.3±5961.7</td>
</tr>
</tbody>
</table>

All values are described as mean±S.D. ANOVA for repeated measures. *P<0.001; effect of session (resting or exercise). No difference between two body type groups.
intake from the endocrine cells (L cells) in the distal gastrointestinal tract before nutrients have reached this location (Adrian et al. 1985), suggesting the existence of unknown neural or endocrine mechanisms by which PYY release is regulated. Similar mechanisms might also participate in the control of hormone release during exercise. Third, we recruited a small number of subjects in the present study, and could not estimate precise statistical power. A large population study will be necessary to confirm the present results. Furthermore, it will be worthwhile investigating physiological association of gut hormone changes induced by moderate intensity exercise with EB regulation in different population because prevalece and definition of obesity and pathophysiological involvement of BMI with obese-related diseases are likely to vary among countries and ethnicities (Yamamoto et al. 2002).

In conclusion, our findings showed that in obese young adults, a single bout of moderate exercise produced significantly negative EB compared with that in age-matched control subjects of normal weight despite similar increases in plasma PYY and GLP-1 in the two groups. These findings suggest that short-term EB during a single bout of exercise might be regulated not by increased amounts of gut hormones per se. Although, the physiological involvement of exercise-induced hormonal changes in EB during exercise has recently been examined (Martins et al. 2007), our findings suggest that it is also important to consider multiple other factors that modify the effects of anorexigenic gut hormones in elucidating the mechanisms by which EB is regulated during and after exercise and developing effective programs for weight management.

Declaration of interest

The authors declare that they have no conflict of interest.

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Author contribution statement

All the authors declare that they participated in the acquisition of data, analysis and interpretation of data, and drafting of the manuscript, and that they have seen and approved the final version.

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