RESEARCH

Effects of walking in water on gut hormone concentrations and appetite: comparison with walking on land

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Abstract

The effects of water exercise on gut hormone concentrations and appetite currently remain unclear. The aim of the present study was to investigate the effects of treadmill walking in water on gut hormone concentrations and appetite. Thirteen men (mean ± s.d. age: 21.6 ± 2.2 years, body mass index: 22.7 ± 2.8 kg/m², peak oxygen uptake (VO₂peak): 49.8 ± 7.8 mL/kg per min) participated in the walking in water and on land challenge. During the study period, ratings of subjective feelings of hunger, fullness, satiety and motivation to eat were reported on a 100-mm visual analog scale. A test meal was presented after walking, and energy intake (EI) was calculated. Blood samples were obtained during both trials to measure glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and acylated ghrelin (AG) concentrations. Hunger scores (How hungry do you feel?) were significantly lower during the water trial than during the land trial (P<0.05). No significant differences were observed in EI between water and land trials. GLP-1 concentrations were significantly higher in the water trial than in the land trial (P<0.05). No significant differences were observed in PYY concentrations between water and land trials. AG concentrations were significantly lower in the water trial than in the land trial (P<0.01). In conclusion, changes in gut hormone concentrations during walking in water contribute to the exercise-induced suppression of appetite and provide novel information on the influence of walking in water on the acute regulation of appetite.

Introduction

Appetite and eating behavior are controlled by various peripheral signals that change in response to food intake and act on the hypothalamus and brainstem (1). These feedback signals involve a number of gut hormones, such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and ghrelin (2). The effects of exercise on these hormones have been investigated extensively over the past decade (3, 4). Previous findings suggested that the concentrations of anorexigenic hormones (GLP-1 and PYY) increased, while those of plasma acylated ghrelin (AG) decreased following endurance exercise (5, 6, 7). We also demonstrated that a single bout of aerobic exercise caused significant increases in GLP-1 and PYY plasma concentrations (8, 9, 10). These findings suggest that exercise partly functions as a physiological regulator for hormone release or metabolism and, thus, controls appetite.

Walking in water is endorsed for maintaining and improving physical fitness and health because of the...
properties of water, such as water pressure, buoyancy
and resistance (11). Therefore, walking in water has been
extensively utilized in rehabilitation for various sports
injuries, including disease prevention in terms of health
care. Although other modes of activities such as resistance
exercise (5, 12, 13), sprint interval training (14) and rope
skipping exercise (15) have been evaluated, the effects of
treadmill walking in water on gut hormone concentrations
and appetite remain unclear. Lambert and coworkers (16)
reported no significant differences in energy intake (EI)
or appetite after exercise between swimming and running
trials. In addition, King and coworkers (17) showed that
an acute bout of swimming suppressed AG concentrations
and appetite. However, the mode of exercise employed by
these studies was swimming and anorexigenic hormones
(GLP-1 and PYY) were not examined. Furthermore, there
were discrepancies between the findings of these studies.
A clearer understanding of the effects of treadmill walking
in water on gut hormone concentrations and appetite
may contribute to exercise guidance for maintaining and
improving body weight and mass for health.

Therefore, the aim of the present study was to
investigate the effects of treadmill walking in water on gut
hormone concentrations and appetite and compare them
to those when walking on land.

Materials and methods

Participants
Twenty-one young male subjects were recruited. All
subjects were lifelong non-smokers with a reported stable
weight and the lack of any special type of diet for the
previous 6 months. None had any history of infectious
disease for at least a 1-month period preceding this
study, and none were taking medication. Subjects with
a history of gastrointestinal, endocrine, cardiovascular
or psychological diseases or type 1 or 2 diabetes were
excluded. All subjects provided written informed consent
for participation in the study, which was approved by the
Ethics Committee of Morinomiya University of Medical
Sciences (Admitting No. 2014-053).

Design
Participants were randomly assigned to participate in one
of the two studies. In study 1, thirteen men (mean ± s.d.
age: 21.6 ± 2.2 years, body mass index: 22.7 ± 2.8 kg/m²,
peak oxygen uptake (VO₂peak): 49.8 ± 7.8 mL/kg per min)
participated in the walking in water and on land challenge.
In study 2, eight men (mean ± s.d. age: 19.5 ± 1.8 years, body
mass index: 22.4 ± 1.3 kg/m², VO₂peak: 53.2 ± 7.1 mL/kg
per min) participated in the water immersion (WI)
challenge. Studies 1 and 2 recruited different subjects.
Subjects were habitually active, but were not trained
athletes, with most individuals typically participating in
games and activities such as soccer, baseball and basketball
on a regular basis at a recreational level. In order to control
physical activity on the days prior to and on the mornings
of the 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%
carbohydrates, 10–20% protein and 20–30% fat (energy
percent) and recorded all food and drink consumed 1 week
prior to and throughout the study period. Dietary records
were analyzed using a computerized nutritional analysis
system (Excel Eiyoukun, Kenpakusha, Tokyo, Japan). All
dietary assessments were performed by experimental
measurers and supervised by researchers. Prior to the
main trial days, each subject attended the laboratory
for a preliminary examination in which anthropometric
measurements (height and body weight) were recorded.
We also measured VO₂peak during the incremental
treadmill protocol as an index of cardiorespiratory fitness
and to select the pace in the walking in water and on land
trials. This test consisted of 3-min stages with the speed of
the treadmill being progressively increased at each stage
by 1–2 km/h until voluntary exhaustion. Respiratory data
during the experiments were recorded using an automatic
breath-by-breath respiratory gas analyzing system (ARCO-
2000, Arcosystem, Chiba, Japan) consisting of a flow meter
for the hot wire in the face mask, an O₂ analyzer made
from a zirconium element and an infrared CO₂ analyzer.

Study 1: Effects of walking in water
All subjects took part in two experimental trials (walking in
water and on land trials) at least 7 days apart. The order of
the two trials was randomized across subjects. The design
of the experimental session is shown diagrammatically
in Fig. 1. Room temperature was set at 25°C in two
experimental trials. Subjects came to the laboratory at
08:30 h and, after a 10-min rest period, a cannula was
inserted into the antecubital vein, and a fasting venous
blood sample (baseline; t=0) was taken (20 mL). Exercise
commenced at 09:00 h, with subjects completing 30 min
of treadmill walking at a speed equivalent to 50% of their
VO₂peak. During this exercise, subjects wore a heart rate
(HR) monitor and breathed through a face mask into the automatic breath-by-breath respiratory gas analyzing system (ARCO-2000, Arcosystem) described previously. Experimental measurers made careful adjustments to coincide the speed to 50% of their VO_{2peak} from the data of the automatic breath-by-breath respiratory gas analyzing system. Subjects were immersed to chest depth (xiphoid process) on an underwater treadmill (AquaCiser Underwater Treadmill System, Ferno-Washington, Inc., Ohio, USA) in thermoneutral water (34°C) during walking in water. Craig and Dvorak (18) recorded rectal temperature during WI and recommended 34°C as the thermoneutral temperature at rest. At this temperature setting, neither coldness nor shivering was reported by any of the participants. Blood samples were obtained after the end of the exercise (exercise condition; t=30) and 30 min after the exercise had finished (t=60). In addition, during the study period (t=0, 30 and 60 min), ratings of subjective feelings of hunger, fullness, satiety and motivation to eat were reported on a 100-mm visual analog scale (19) and the feeling of dyspnea and leg fatigue were assessed according to Borg scales (20).

At 10:00 h (t=60 min), a test meal (instant pasta: 7.9% protein, 44.6% fat and 47.5% carbohydrates (energy percent)) was provided and subjects were instructed to eat as much as they liked until satisfied, as described previously (8, 9, 10). In order to exclude the possibility that the amount of food eaten depended on its palatability, we asked all subjects of the 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 trials, subjects and experimenters were instructed to abstain from talking about the meal. Participants were not overly informed that the true purpose of the present study was to assess feeding responses where possible until they had completed the protocol. Water was freely available during trials. After consumption of the test meal, any remaining food was weighed and the amount eaten was subtracted from the premeal value to obtain the total amount of food ingested. Absolute ELs from the test meal in both trials were then calculated from the amount of food eaten (1.15 kcal/g).

**Study 2: Effects of WI (supplemental protocol)**

We investigated the effects of WI on gut hormone concentrations and appetite. Subjects came to the laboratory at 08:30h and, after a 10-min rest period, a cannula was inserted into the antecubital vein, and a fasting venous blood sample (without WI; t=0) was taken (20mL). The subject rested in the water tank in a standing position with WI (34°C) for 30 min. The subject’s body was immersed in water up to the level of the chest (xiphoid process). Changes in thoracic volume were estimated using impedance plethysmography (21). Subjects wore an HR monitor and breathed through a face mask into the automatic breath-by-breath respiratory gas analyzing system (ARCO-2000, Arcosystem). Blood samples were obtained after the end of WI (WI condition; t=30). In addition, during the study period (t=0 and 30 min), ratings of subjective feelings of hunger, fullness, satiety and motivation to eat were reported on a 100-mm visual analog scale (19).

**Blood sampling**

Blood samples were immediately transferred into disodium EDTA-treated tubes to measure plasma gut hormones, glucose, insulin and IL-6. Aprotinin, a potent protease inhibitor, was added to samples at a concentration of 500kIU/mL for the measurement of AG. Test tubes were then centrifuged at 1308 g at 4°C for 15 min immediately after collection, and plasma samples were stored at −80°C for later analyses. Hematocrit was measured using Celltac alpha (Nihon Kohden Inc., Tokyo, Japan). Glucose was measured using the enzymatic reference method with hexokinase. Insulin was assessed by the fully automated chemiluminescence method (CLIA). Plasma IL-6 (Human
Walking in water and gut hormones

1.34 ± 0.5  
Water  
s.d.  
*Significance
0.45 ± 0.4

Values less than 0.05

Pearson’s correlation coefficient. 
P

hunger scores were assessed by a simple correlation using 
addition, the relationships among each gut hormone and 
comparisons (Tukey–Kramer test) were performed. In 
significance was detected, 
multiple pair-wise 
post hoc  
of variance with repeated measures was performed. If

plasma gut hormones and VAS scores, a two-way analysis 
the effects of trials and condition on the concentrations of 
assessed using Student’s paired  
t-test. In order to examine

and leg fatigue scores and central blood volume were 
parameters, expired gas data, HR, CVRR, dyspnea feeling 
which are often used to indicate parasympathetic nervous 
activity. The effects of the trial on EI, biochemical 
parameters, expired gas data, HR, CVRR, dyspnea feeling and leg fatigue scores and central blood volume were 
assessed using Student’s paired t-test. In order to examine 
the effects of trials and condition on the concentrations of 
plasma gut hormones and VAS scores, a two-way analysis 
of variance with repeated measures was performed. If 
significance was detected, post hoc multiple pair-wise 
comparisons (Tukey–Kramer test) were performed. In 
addition, the relationships among each gut hormone and 
hunger scores were assessed by a simple correlation using 
Pearson’s correlation coefficient. P-Values less than 0.05 
were considered to be significant.

Results

Study 1: Effects of walking in water

Exercise characteristics
No significant changes were observed in hematocrit over time during exercise. Therefore, hemoconcentration was unlikely to have occurred during the exercise performed in the present study (data not shown). No significant differences were noted in the mean values of VO2, %VO2peak and respiratory exchange ratio (RER) during walking between water and land trials (Table 1). Mean

<table>
<thead>
<tr>
<th>VO2 (L/min)</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.67±0.11</td>
<td>1.64±0.12</td>
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<th>Water</th>
<th>Significance</th>
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<td>51.6±4.8</td>
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<th>Land</th>
<th>Water</th>
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<td>118±6.7</td>
<td>111±4.1</td>
<td>*</td>
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<table>
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<th>CVRR (%)</th>
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<th>Water</th>
<th>Significance</th>
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<td>2.5±2.4</td>
<td>3.9±2.0</td>
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<tr>
<th>Dyspnea feeling (score)</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
</tr>
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<tr>
<td>3.6±1.3</td>
<td>3.4±1.6</td>
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<table>
<thead>
<tr>
<th>Leg fatigue (score)</th>
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<th>Water</th>
<th>Significance</th>
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<tr>
<td>3.4±1.3</td>
<td>6.7±1.6</td>
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<table>
<thead>
<tr>
<th>Walking speed (km/h)</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>6.6±0.9</td>
<td>6.0±0.5</td>
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<tr>
<th>Biochemical parameters</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>91.6±8.1</td>
<td>90.4±8.9</td>
<td>NS</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin (μU/mL)</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7±2.8</td>
<td>3.6±2.4</td>
<td>NS</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>IL-6 (pg/mL)</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45±0.4</td>
<td>1.34±0.5</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means±s.d.
*P<0.05, **P<0.01; water vs land trials.

CVRR, coefficient of variation of R–R intervals; HR, heart rate; IL-6, interleukin-6; RER, respiratory exchange ratio; VO2, oxygen uptake.

HR values during walking were significantly lower in the water trial than in the land trial (P<0.05; Table 1). Furthermore, CVRR during walking was significantly higher in the water trial than in the land trial (P<0.05; Table 1). No significant differences were observed in the feeling of dyspnea after walking between water and land trials (Table 1). In contrast, leg fatigue after walking was significantly higher in the water trial than in the land trial (P<0.05; Table 1). Walking speed was significantly slower in the water trial than in the land trial (P<0.01; Table 1).

EI and measures of appetite
No significant differences were observed in EI after walking between the two trials (Fig. 2A). Hunger scores (How hungry do you feel?) were significantly lower when walking in water than on land (P<0.05; Fig. 2B). No significant differences were observed in fullness (How full do you feel?), satisfaction (How satisfied do you feel?) or motivation to eat (How much do you feel you can eat?) scores between the two trials (data not shown).

Gut hormone concentrations
No significant differences were observed in GLP-1 or PYY concentrations after walking between water and land trials (Fig. 3A–C). However, mean AUC values for GLP-1 were significantly higher in the water trial than in the land trial (P<0.05; Fig. 3B).

Table 1  Exercise characteristics and biochemical parameters with walking in water and on land.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Land</th>
<th>Water</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>VO2 (L/min)</td>
<td>1.67±0.11</td>
<td>1.64±0.12</td>
<td>NS</td>
</tr>
<tr>
<td>% VO2peak (%)</td>
<td>51.6±4.8</td>
<td>50.7±5.2</td>
<td>NS</td>
</tr>
<tr>
<td>RER</td>
<td>0.97±0.05</td>
<td>0.99±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>118±6.7</td>
<td>111±4.1</td>
<td>*</td>
</tr>
<tr>
<td>CVRR (%)</td>
<td>2.5±2.4</td>
<td>3.9±2.0</td>
<td>*</td>
</tr>
<tr>
<td>Dyspnea feeling (score)</td>
<td>3.6±1.3</td>
<td>3.4±1.6</td>
<td>NS</td>
</tr>
<tr>
<td>Leg fatigue (score)</td>
<td>3.4±1.3</td>
<td>6.7±1.6</td>
<td>*</td>
</tr>
<tr>
<td>Walking speed (km/h)</td>
<td>6.6±0.9</td>
<td>6.0±0.5</td>
<td>**</td>
</tr>
<tr>
<td>Biochemical parameters Glucose (mg/dL)</td>
<td>91.6±8.1</td>
<td>90.4±8.9</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>3.7±2.8</td>
<td>3.6±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.45±0.4</td>
<td>1.34±0.5</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are presented as means±s.d.
*P<0.05, **P<0.01; water vs land trials.

CVRR, coefficient of variation of R–R intervals; HR, heart rate; IL-6, interleukin-6; RER, respiratory exchange ratio; VO2, oxygen uptake.

No significant changes were noted in the mean values of %VO2peak and respiratory exchange ratio (RER) during walking between the two trials (Fig. 3B).
Mean AG concentrations were significantly lower when walking in water than on land ($P<0.05$; Fig. 3E). Furthermore, mean AUC values for AG were significantly lower in the water trial than in the land trial ($P<0.01$; Fig. 3F).

**Glucose metabolism and IL-6**

No significant differences were observed in glucose or insulin concentrations between the two trials (Table 1). IL-6 concentrations during walking were significantly higher in the water trial than in the land trial ($P<0.05$; Table 1).
Relationships among gut hormone levels and hunger scores

An examination of the relationships among plasma AG concentrations and hunger rating scores at individual time points (t = 0, 30 and 60) revealed a correlation at t = 30 (after the end of walking) in the walking on land and in water (r = 0.631, P < 0.01; Fig. 4) trial, but at no other points in the walking in water and on land trial. Plasma GLP-1 and PYY concentrations did not correlate with hunger rating scores at any time point in either trial (data not shown).

Study 2: Effects of WI (supplemental protocol)

No significant differences were noted in the mean values of VO$_2$, RER or gut hormone concentrations (Table 2). There was a 12.9 ± 1.7% increase in thoracic admittance (P < 0.001; Table 2) corresponding to an increase in central blood volume during WI, whereas HR was significantly decreased during WI by 15.8 ± 6.9% (P < 0.01; Table 2). No significant differences were noted in the ratings of subjective feelings of hunger, fullness, satiety and motivation to eat during WI (Table 2).

Discussion

The objective of the present study was to investigate the effects of treadmill walking in water on gut hormone concentrations and appetite and compare them to those when walking on land. The following results were obtained: (1) hunger scores were significantly lower when walking in water than on land; (2) GLP-1 concentrations were significantly higher and AG concentrations were significantly lower when walking in water than on land; and (3) a positive correlation was observed between plasma AG concentrations and hunger scores after the end of walking in water and on land.

EI and appetite responses to walking in water have not previously been examined, and no significant differences were observed in EI after walking between water and land trials in this study. A recent systematic review reported that exercise did not increase or decrease absolute EI following an exercise task (22). Swimming did not affect energy or macronutrient intake between swimming and resting trials (17). In addition, Lambert and coworkers (16) reported no significant differences in EI after exercise between swimming and running trials, which is consistent with the present study. On the other hand, the present results suggest that walking in water caused a significantly greater reduction in hunger sensations than walking on land. One possible explanation for this inhibition is the difference in energy expenditure and intensity between water and land trials. However, energy metabolism in the water and land trials matched, with no significant difference being observed in VO$_2$. In addition, no significant differences were noted in the feeling of dyspnea after walking between water and land trials. Another possibility is that inhibitory responses are linked to the changes observed in gut hormone concentrations during walking in water. Lambert and coworkers (16) reported no significant differences in the hunger scale, which is inconsistent with the present results. Although

Table 2  Effects of water immersion

<table>
<thead>
<tr>
<th></th>
<th>Without WI</th>
<th>With WI</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_2$ (mL/min)</td>
<td>332 ± 51</td>
<td>348 ± 54</td>
<td>NS</td>
</tr>
<tr>
<td>RER</td>
<td>0.81 ± 0.05</td>
<td>0.82 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>69.6 ± 6.9</td>
<td>58.5 ± 6.3</td>
<td>**</td>
</tr>
<tr>
<td>CBV ($5 \times 10^{-3}$)</td>
<td>42.8 ± 3.2</td>
<td>48.3 ± 3.8</td>
<td>***</td>
</tr>
<tr>
<td>Gut hormones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 (pmol/mL)</td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>PYY (pmol/mL)</td>
<td>1.8 ± 0.6</td>
<td>1.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>AG (fmol/mL)</td>
<td>18.6 ± 5.5</td>
<td>17.8 ± 5.3</td>
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</tr>
<tr>
<td>Appetite</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hunger (mm)</td>
<td>70.0 ± 20.4</td>
<td>61.1 ± 20.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>22.6 ± 19.5</td>
<td>23.8 ± 21.8</td>
<td>NS</td>
</tr>
<tr>
<td>Satiety (mm)</td>
<td>21.7 ± 23.7</td>
<td>20.9 ± 24.7</td>
<td>NS</td>
</tr>
<tr>
<td>Motivation to eat (mm)</td>
<td>82.4 ± 21.7</td>
<td>83.9 ± 22.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are presented as means ± s.d.
***P < 0.001, ** P < 0.01.
AG, acylated ghrelin; CBV, central blood volume; HR, heart rate; RER, respiratory exchange ratio; VO$_2$, oxygen uptake; WI, water immersion.

Figure 4

Relationships between acylated ghrelin concentrations and hunger scores at t = 30 (after the end of walking) in the walking in water (○) and on land (●) trials.
we cannot provide a definite explanation for this discrepancy, the different characteristics of the subjects enrolled for the study may have affected this outcome. In the study by Lambert and coworkers (16), well-trained competitive male triathletes were enrolled. Therefore, well-trained triathletes may have different responses to aquatic exercise than non-triathletes because of specific metabolic and endocrine (such as appetite-related hormones) adaptations to aquatic exercise. However, to the best our knowledge, this issue has not yet been investigated; thus, our hypothesis remains speculative. Future research is needed to clarify this point.

A systematic review recently reported that acute exercise increased GLP-1 concentrations (23). We also demonstrated that a single bout of aerobic exercise caused significant increases in GLP-1 plasma concentrations (8, 9, 10). These findings suggest that exercise partly functions as a physiological regulator for GLP-1 release or metabolism and, thus, leads to appetite control. We herein demonstrated that AUC values for GLP-1 concentrations were significantly higher when walking in water than on land. Previous studies reported that increases in the plasma concentrations of GLP-1 (24, 25, 26, 27, 28) were mediated by a neural pathway, regardless of exercise. The vagus nerve efferent pathway was examined for its global mediated by a neural pathway, regardless of exercise. The vagus nerve efferent pathway was examined for its global effects on basal GLP-1 secretion and represented the main parasympathetic nerve innervating the proximal portion of the gastrointestinal tract (27, 29, 30). HR during exercise in water was also lower than that on land at the same VO₂ and one major mechanism for this phenomenon is vagal nerve activity (31). Background vagal nerve activity in the present study was higher for walking in water than on land. CVRR during exercise was significantly higher in the water trial than in the land trial. These results indicated that the higher AUC values for GLP-1 concentrations when walking in water than on land were mediated by activation of the vagal nerve. Another possible explanation for this phenomenon is the difference in IL-6 kinetics between water and land trials. In order to clarify whether IL-6 was required for this exercise-induced increase in GLP-1, Ellingsgaard and coworkers (32) subjected IL-6 knockout mice to a bout of exercise and found that plasma GLP-1 concentrations did not increase in response to this manipulation. Although the present study demonstrated that GLP-1 and IL-6 concentrations had the same patterns during the 60-min water/land trials, GLP-1 concentrations did not correlate with IL-6 concentrations at any time point in either trial (data not shown). Further studies are needed in order to elucidate the interplay between the two mechanistic pathways (neural and humoral) underlying increases in GLP-1 plasma concentrations during exercise. In addition, total GLP-1 was measured rather than active GLP-1 in the present study. Since we did not add a dipeptidyl peptidase 4 (DPP-4) inhibitor, we were unable to measure active GLP-1 concentrations. A previous study demonstrated that active and total GLP-1 concentrations showed the same pattern during the glucose tolerance test (33). Therefore, these findings prompted us to speculate that total GLP-1 concentrations may reflect active GLP-1 responses to exercise. Future studies incorporating a DPP-4 inhibitor need to be conducted in order to enable the assessment of active GLP-1 concentrations because previous findings have suggested that total GLP-1 concentrations reflect changes in active GLP-1 concentrations.

Plasma PYY concentrations also generally increase with exercise, similar to GLP-1 (4, 23). In the present study, PYY concentrations increased during the walking in water and on land trials, which is consistent with previous findings (4, 5, 7, 8, 9, 10, 23). However, no significant differences were observed in PYY concentrations between the two trials; therefore, the physiological mechanisms responsible for this phenomenon currently remain unknown. We previously demonstrated that while increases in PYY concentrations were dependent on exercise intensity, GLP-1 concentrations were similar between high- and moderate-intensity exercise sessions (9). Of particular interest in the present study were the different results obtained between GLP-1 and PYY despite these hormones having the same secretory cells and anorexigenic functions, similar to our previous findings (9). In other words, the physiological mechanisms underlying the secretion of GLP-1 and PYY during exercise were caused by a different mechanism. Collectively, the present results appear to provide a novel insight into the mechanisms underlying exercise-induced changes in GLP-1 and PYY concentrations in humans.

Ghrelin is secreted into the circulation in which it is present in two forms: acylated and unacylated (34, 35). The appetite regulation of ghrelin is now understood to be chiefly influenced by AG (3, 34). A recent meta-analysis found that exercise may influence appetite by suppressing AG concentrations (23). In addition, AG was previously shown to be suppressed during swimming (17). We herein demonstrated that walking in water decreased AG plasma concentrations. The positive correlation between plasma AG concentrations and hunger scores after the end of walking in water and on land also indicated that AG concentrations were more likely to be high in those with higher hunger scores.
scores. Broom and coworkers (36) also found that decreases in AG concentrations during running exercise positively correlated with decreases in hunger scores. One of the possible interpretations of this result is that in this study setting, AG was more closely involved in ‘exercise-induced anorexia’ (43). These findings provide novel information regarding the influence of walking in water on the acute regulation of appetite and AG. In addition, we speculated that AG concentrations were significantly lower in the water trial than in the land trial due to an increase in leg fatigue, and IL-6 may be involved in resistance exercise because of water viscosity and resistance as well as differences in muscle activity during walking in water. Broom and coworkers (5) reported that treadmill running suppressed AG, whereas resistance exercise promoted it.

There were some potential limitations to the present study. Subjects in the present study were young, healthy males; therefore, we do not know whether our results may be generalized to other populations such as females, the elderly or overweight and obese individuals. We previously demonstrated that gut hormone (AG, GLP-1 and PYY) concentrations showed the same pattern during aerobic exercise in obese and non-obese subjects (10). Therefore, we speculated that similar results may be obtained from overweight and obese individuals in the present study. A sex difference had a significant influence on appetite sensations (37). In addition, gut hormones and appetite respond differently to exercise in males and females (38, 39). This sex difference is prominently attributed to the menstrual cycle (40). Therefore, we focused on males and excluded females. Further studies are needed in order to clarify whether there is a sex difference in gut hormones and appetite after walking in water. Furthermore, body temperature was not measured in the present study. Previous studies demonstrated that changes in body temperature during and after exercise may affect appetite, hormone circulation and food intake. Shorten and coworkers (41) demonstrated that EI was greater after exercise at a neutral temperature (25°C) than under resting conditions, but was similar between rest and exercise in the heat (36°C). Other studies examined the effects of exercise performed in a cold environment on appetite. White and coworkers (42) reported that EI was significantly higher after exercise in cold water (20°C) than in neutral water (33°C) and resting trials. In the present study, body temperature may have differed between the two experimental trials (walking in water and on land trials) even though the temperature was the same as air temperature (25°C) when walking in water and on land. Differences in body temperature when walking in water and on land may have contributed to differences in hunger and hormone concentrations between the two conditions. In the future, a clearer understanding of the relationship between body temperature and gut hormone concentrations during walking in water may assist in the development of new exercise programs to prevent and treat lifestyle diseases such as obesity, type 2 diabetes and hyperlipidemia. Another limitation is that the time of observations was short in the present study. Most studies indicated that individuals did not compensate for energy expended during exercise in the immediate hours after exercise by altering food intake (22). In addition, subjective feelings of hunger are suppressed during and after exercise; however, this suppression was short-lived (43). In the future, appetite sensations need to be measured for a short time after the meal in order to examine differences in the satiating effects of a meal following exercise in water vs. that on land. Additionally, we cannot rule out the possibility that cognitive or environmental factors affected our results even though we attempted to carefully exclude confounding variables based on the choice of study design. In previous studies on appetite, subjects were instructed to eat ad libitum (23). However, under these circumstances, food intake may be biased by cognitive factors such as the belief that ‘food is a reward for exercise’ (7). In the present study, a common test meal of noodles was prepared in order for subjects to be unaware of food intake during the test (8, 9, 10).

In conclusion, we herein demonstrated that GLP-1 concentrations were significantly higher and AG concentrations were significantly lower when walking in water than on land. These results suggest that changes in gut hormone concentrations during walking in water contribute to the exercise-induced suppression of appetite. Our results may contribute to exercise guidance for maintaining and improving body weight and mass for health.

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

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Author contribution statement
Shin-ya Ueda: designed the experiments, developed analyses and drafted and revised the manuscript. Hidehiro Nakahara, Eriko Kawai, Tatuya Usui and Shinharo Tsuji: collected, analyzed and provided initial interpretations of the data. Tadayoshi Miyamoto: collected and analyzed the data, and reviewed and revised the manuscript.

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