

Exercise induced suppression of acylated ghrelin in humans

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Running Head

Exercise and acylated ghrelin

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ABSTRACT

Ghrelin is an orexigenic hormone secreted from endocrine cells in the stomach and other tissues. Acylation of ghrelin is essential for appetite regulation. Vigorous exercise induces appetite suppression but this does not appear to be related to suppressed concentrations of total ghrelin. This study examined the effect of exercise and feeding on plasma acylated ghrelin and appetite. Nine males aged 19 to 25 y participated in two, 9-h trials (exercise and control) in a random crossover design. Trials began at 08:00 h in the morning after an overnight fast. On the exercise trial subjects ran for 60 min at 72% of maximum oxygen uptake between 08:00 and 09:00 h. After this, they rested for 8 h and consumed a test meal at 11:00 h. On the control trial subjects rested for 9 h and consumed a test meal at 11:00 h. Area under the curve values for plasma acylated ghrelin concentration (assessed from venous blood samples) were lower over the first three hours and the full nine hours of the exercise trial compared with the control trial: 317 ± 135 versus 510 ± 186 $\text{pg}\cdot\text{mL}^{-1}\cdot 3$ h and 917 ± 342 versus 1401 ± 521 $\text{pg}\cdot\text{mL}^{-1}\cdot 9$ h (mean \pm SEM) respectively ($P < 0.05$). Area under the curve values for hunger (assessed using a visual scale) were lower over the first three hours of the exercise trial compared with the control trial ($P = 0.013$). These findings demonstrate that plasma acylated ghrelin concentration and hunger are suppressed during running.

Key Words: running, appetite, hunger

INTRODUCTION

Ghrelin is a 28-amino acid peptide hormone which stimulates the release of growth hormone from the pituitary (29). Ghrelin is secreted primarily from cells within the stomach but ghrelin is synthesized and secreted in many other tissues including the small intestine, pancreas, hypothalamus, cardiomyocytes, chondrocytes and placenta (9, 30). Plasma ghrelin concentrations rise prior to meals and decrease following meals suggesting that ghrelin is orexigenic (appetite stimulating) (12, 13). This suggestion is supported by the findings that intracerebroventricular ghrelin administration stimulates feeding in rats (37, 51) and intravenous ghrelin infusion increases food intake in humans (16, 50).

Several studies have investigated the influence of high intensity aerobic exercise bouts on plasma ghrelin concentration (7, 14, 24, 25, 32, 43, 49). Most of these studies indicate that a single session of aerobic exercise has no influence on ghrelin concentrations (7, 14, 24, 25, 32, 43) although one study reported that serum ghrelin levels are suppressed for up to an hour after the cessation of exercise (49). In contrast, one study has reported that plasma ghrelin concentrations are increased during three hours of moderate intensity exercise but it is uncertain whether this is a true effect of exercise because this study did not include a control trial (11). Furthermore, fasting ghrelin concentrations appear to be unaffected by exercise training in the absence of concurrent weight loss (33). This indicates that ghrelin may be a sensor of negative energy balance as suggested previously (41).

A limitation of research concerning exercise and ghrelin is that all of the studies performed so far have measured total ghrelin (7, 11, 14, 24, 25, 32, 43, 49). Ghrelin

exists in non-acylated and acylated forms with the majority (80 to 90%) being non-acylated (22, 23). Acylation is thought to be essential for ghrelin to bind to the growth-hormone-secretagogue receptor and to cross the blood brain barrier (29, 36). In humans non-acylated ghrelin does not possess the pituitary and pancreatic activity of acylated ghrelin (6). Therefore, non-acylated ghrelin is considered to be unimportant for appetite regulation although it may have other functions such as inhibiting cell proliferation (10) and stimulating adipogenesis (48).

Relatively few studies have measured acylated ghrelin because it is less stable than total ghrelin (23) creating practical difficulties in sample collection and processing. Two studies have shown that acylated ghrelin is suppressed after mixed meals and provide evidence that macronutrient composition may affect the extent of this suppression (1, 17). Three studies have simultaneously measured acylated and total ghrelin responses to food consumption (2, 34, 23). These studies have confirmed that acylated ghrelin is suppressed after food intake and one of these studies found that acylated ghrelin responds more rapidly and dynamically than total ghrelin in response to glucose ingestion (23). In view of this latter finding and bearing in mind that appetite is suppressed following exercise (26, 27) we decided to re-investigate the influence of exercise on ghrelin by specifically measuring acylated ghrelin.

Therefore, the primary purpose of the present study was to determine plasma acylated ghrelin concentrations and hunger ratings during and following an intense bout of treadmill running. We hypothesized that intense treadmill running would cause a temporary suppression of hunger and that this would be associated with reduced concentrations of plasma acylated ghrelin. We also assessed the acylated ghrelin

response to feeding since limited information is available in this regard. Finally, we measured the concentrations of plasma glucose and insulin during and after exercise and feeding because glucose and insulin have suppressive effects on ghrelin and are therefore important for ghrelin regulation (19, 35, 44).

SUBJECTS AND METHODS

Participants

After approval from the University Ethical Advisory Committee, nine healthy Caucasian males aged 19 to 25 y gave their written informed consent to participate in this study. Most of the volunteers were sports science students studying at Loughborough University. All participants were physically active and most were involved in competitive sports such as soccer, rugby, tennis and hockey. Prior training status was not a prerequisite for the study but the physical demands of the study ensured that all of the subjects were reasonably fit. The physical characteristics of the subjects (mean \pm SEM) were as follows: age 21.2 ± 0.7 y, height 1.81 ± 0.02 m, body mass 72.6 ± 2.0 kg, BMI 22.2 ± 0.7 kg·m⁻², waist circumference 78.3 ± 1.7 cm, maximum oxygen uptake 63.3 ± 2.0 mL·kg⁻¹·min⁻¹ (4.56 ± 0.09 L·min⁻¹).

Experimental Design

Prior to the main trials each subject attended the laboratory for a preliminary session in which anthropometric data (height, weight, waist circumference) were collected and two preliminary exercise tests were performed as follows: 1) submaximal-incremental treadmill running test, 2) maximum oxygen uptake treadmill running test. There was a 20 to 30 min rest interval between the exercise tests. After the preliminary session subjects were given at least a week to recover from the exercise testing before the main trials began. There were two main trials (exercise and control) and these were performed in a randomized order. The exercise trial involved a one hour run followed by an eight hour rest period while the control trial involved nine hours of rest. A meal was consumed three hours after the start of each trial and blood samples were collected throughout each trial for the determination of plasma acylated

ghrelin, glucose and insulin concentrations. In addition hunger ratings were recorded at intervals throughout each trial using a visual scale.

Anthropometry

Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Hamburg, Germany). Weight was measured to the nearest 0.01 kg using a balance beam scale (Avery, Birmingham, U.K.). BMI was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was determined as the widest part of the torso between the xiphoid process of the sternum and the iliac crest.

Submaximal-incremental treadmill running test

After treadmill familiarization subjects completed a submaximal-incremental treadmill running test to determine the relationship between running speed and oxygen consumption while running on a level motorized treadmill (RUNRACE, Technogym, Gambettola, Italy). The test was designed to exercise subjects through a range of intensities from moderate to vigorous but not maximum. The test was 16-min in duration and was continuous in nature but was divided into four, four min stages. The initial running speed was set between 7 and 8 km/h depending upon each subject's fitness level. Treadmill speed was increased by 1 or 1.5 km/h at the end of each four min period depending upon each subject's fitness level. Expired air samples were collected into Douglas bags for the final min of each four min stage for the determination of oxygen consumption and carbon dioxide production. Four min stages were used to ensure that subjects were in steady state during expired air collection periods. Heart rate was monitored continuously throughout the test using short-range telemetry (Polar A3, Kempele, Finland). Ratings of perceived exertion (5) were

assessed simultaneously with the expired air collections during the tests. At the end of the test the oxygen consumption at each stage was plotted against the running speed at each stage to illustrate the running speed-oxygen consumption relationship.

Maximum oxygen uptake test

Subjects were given 20 to 30 minutes to recover from the submaximal treadmill test before they began the maximum oxygen uptake test. Maximum oxygen uptake was measured directly using an incremental uphill protocol at a constant speed until the subjects reached volitional fatigue (46). The test was designed so that subjects would reach volitional fatigue within 10 to 12 min as recommended by Taylor et al (46). The initial incline of the treadmill was set at 3.5%. Thereafter treadmill gradient was increased by 2.5% every 3 min. Thus, treadmill incline was 3.5% during min 1 to 3, 6% during min 4 to 6, 8.5% during min 7 to 9 and 11% during min 10 to 12. Expired air samples were collected into Douglas bags between min 1:45 and 2:45 of each three min stage and during the final min of the test which occurred when subjects signaled that they could only continue for one more min. Heart rate was monitored throughout these tests using short-range telemetry (Polar A3, Kempele, Finland). Ratings of perceived exertion (5) were assessed simultaneously with the expired air collections during each test.

At the end of the maximum oxygen uptake test oxygen consumption and carbon dioxide production were determined from each expired air sample and the highest value was accepted as the maximum oxygen uptake. Criteria used to confirm a true maximum value included two or more of the following: 1) heart rate within ± 10 $\text{b}\cdot\text{min}^{-1}$ of age-predicted maximum heart rate, 2) a respiratory exchange ratio value \geq

1.15, 3) a plateau in oxygen consumption. Once maximum oxygen uptake was determined the oxygen consumption ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) representing 75% of maximum oxygen uptake was calculated. This value was used together with the data obtained in the submaximal-incremental test to estimate the running speed (during level running) eliciting 75% of maximum oxygen uptake. This running speed was used in the main trial.

Dietary control

Throughout the day before the first main trial, participants weighed and recorded their food intake. Participants then replicated this food intake during the day prior to the second main trial. Participants were asked to remain inactive and to avoid caffeine and alcohol consumption in the 24 h prior to each main trial. On the mornings of the main trials, participants arrived at the laboratory having fasted for a minimum of 10 h (no food or drink except water).

Main Trials

Participants were given at least one week to recover from the preliminary exercise tests before performing two main trials (exercise and control), in a random, crossover design with an interval of at least 7 days between trials. Each main trial began in the morning at approximately 08:00 h and lasted for nine hours (until 17:00 h). At the start of the exercise trial participants ran on the treadmill for 60 min (08:00 to 09:00 h) at a running speed predicted to elicit 75% of maximum oxygen uptake. One min expired air samples were collected at 08:14 to 08:15, 08:29 to 08:30, 08:44 to 08:45 and 08:59 to 09:00 h during the run. Running speed was adjusted after each expired air collection if the oxygen consumption was above or below the predicted value.

After the run, participants rested for 8 h (sitting reading, writing, working at a computer or watching television) and consumed a test meal at 11:00 h. This test meal was consumed within 15 min. Subjects left the laboratory at 17:00 h. On the control trial participants rested for the full nine hours (sitting reading, writing, working at a computer or watching television) from 08:00 to 17:00 h and they consumed an identical test meal to that consumed during the exercise trial at 11:00 h. As in the exercise trial this test meal was consumed within 15 min. A clock was on display in the laboratory throughout the trials and therefore subjects were not devoid of time cues. Environmental temperature and humidity were monitored during the main trials using a hand-held hygrometer (Omega RH85, Manchester, U.K.).

Prior to the start of each trial subjects rested in a semi-supine position while a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Venous blood samples were subsequently collected into pre-cooled 4.9 mL EDTA monovettes (Sarstedt, Leicester, U.K.) at 08:00, 08:30, 09:00, 10:00, 11:00, 11:30, 12:00, 13:00, 14:00, 15:00, 16:00 and 17:00 h. Patency of the cannula was maintained by flushing with a small amount of non-heparinised saline (0.9% w/v Sodium Chloride, Baxter Healthcare Ltd, Norfolk, U.K.) after each collection. The saline waste remaining in the connector tube after flushing was drawn off with a 2 mL syringe immediately before the next blood sample was collected. During the exercise trial at 08:30 h, blood was collected while the participants straddled the treadmill, this took approximately 1 min. All other blood samples were collected whilst subjects lay in a semi-supine position. The EDTA monovettes were spun at 1681 g for 10 min in a refrigerated centrifuge (Burkard, Hertfordshire, U.K.) at 4°C. The plasma supernatant

was then aliquoted into Eppendorf tubes. These were stored at -20°C for analysis of glucose and insulin later.

Separate venous blood samples were drawn into 4.9 mL monovettes at 08:00, 08:30, 09:00, 11:00, 12:00 and 17:00 h for the determination of plasma acylated ghrelin concentration. These monovettes contained EDTA and p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. The monovettes were spun at 1287 g for 10 min in a refrigerated centrifuge at 4°C . The supernatants were then aliquoted into storage tubes and 100 μL of 1 M hydrochloric acid (HCL) was added per mL of plasma. Samples were then spun at 1287 g for 5 min in a refrigerated centrifuge at 4°C before being transferred into Eppendorf tubes. The samples were then stored at -20°C for analysis later.

At each blood sampling point, duplicate 20 μL blood samples were collected into micropipettes for the measurement of hemoglobin concentration and triplicate 20 μL blood samples were collected into heparinised micro hematocrit tubes for the determination of hematocrit. These were used to calculate changes in plasma volume (15).

Analysis of expired air samples

Oxygen consumption and carbon dioxide production were determined from expired air samples using a paramagnetic oxygen analyzer and an infra-red carbon dioxide analyzer respectively (Series 1400; Servomex, Crowborough, East Sussex, U.K.). These analyzers were calibrated prior to analysis using gases of known concentration. Expired air volumes were measured using a dry gas meter (Harvard Apparatus,

Edenbridge, Kent, U.K.) and corrected to standard temperature and pressure (dry). Oxygen consumption and carbon dioxide production values were used to calculate energy expenditure using indirect calorimetry (21).

Assessment of hunger

At the beginning of each main trial participants rated how hungry they felt using a 16-point scale that ranged from 0 'Not Hungry' to 15 'Very Hungry'. Hunger ratings were recorded every 30 min thereafter for 5 h and at hourly intervals after this. A previously validated visual analogue scale was also used to assess hunger (20). The two scales yielded identical findings and only the findings from the 16-point scale will be reported.

Test Meal

The test meal fed to participants at 11:00 h on both trials comprised a cheese, butter and mayonnaise sandwich, crisps, a chocolate bar and a full fat milkshake. The macronutrient content of this meal was as follows: 1.47 g carbohydrate, 0.34 g protein, 0.81 g fat and 60 kJ per kg body mass. This provided 38% of calories as carbohydrate, 10% as protein and 52% as fat. Participants were encouraged to consume the meal within 15 min and kept to the same start and finish times on both trials. Water was available *ad libitum* during trials and the volume and time of ingestion were recorded.

Blood biochemistry

Plasma acylated ghrelin concentrations were determined by enzyme immunoassay (SPI BIO, Montigny le Bretonneux, France supplied by Immuno Diagnostic Systems

(IDS)) using a plate reader (Opsys Microplate Reader, Dynex Technologies Inc., Franklin MA, U.S.). Plasma glucose concentrations were determined by enzymatic, colorimetric methods (Randox Laboratories Ltd., County Antrim, U.K.) with the aid of an automated centrifugal analyzer (Cobas Mira Plus; Roche, Basel, Switzerland). Plasma insulin concentrations were determined by a solid-phase ¹²⁵I radioimmunoassay available in a commercial kit (MP Biomedicals, Orangeburg, NY, U.S.) using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL, U.S.). To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within batch coefficients of variation for the assays were as follows: acylated ghrelin 6.6%, insulin 7.4% and glucose 1.8%.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Science (SPSS) software version 12.0 for Windows (SPSS Inc, Chicago, IL, U.S.). Plasma acylated ghrelin, glucose and insulin area under the concentration versus time curves were calculated using the trapezoidal rule. Area under the curve (AUC) values for hunger versus time were also assessed using the same method. Student's *t*-tests for correlated data were used to assess differences between fasting values and between AUC values for acylated ghrelin, glucose, insulin and hunger on the control and exercise trials. Repeated measures, two-factor ANOVA was used to examine differences between the two trials over time for acylated ghrelin, glucose, insulin, hunger, body mass and plasma volume change. Where appropriate, post-hoc pair wise comparisons were performed using the Bonferroni method. The Pearson product moment correlation coefficient was used to examine relationships between variables. Statistical significance was accepted at the 5% level. Adjustment of values for changes in

plasma volume did not alter the statistical findings and hence for simplicity the unadjusted values are reported. Results are given as mean \pm SEM unless otherwise stated.

RESULTS

Responses to treadmill running

The mean percentage of maximum oxygen uptake elicited during exercise was $72 \pm 2.0\%$ and the mean respiratory exchange ratio was 0.94 ± 0.01 . Gross energy expenditure during exercise was 3.9 ± 0.2 MJ with $15 \pm 4\%$ of energy provided from fat and $85 \pm 4\%$ of energy provided from carbohydrate. Average heart rate during exercise was 179 ± 2 beats/min and median rating of perceived exertion (RPE) was 15 i.e. 'hard' (range 13-16).

Body mass and fluid consumption

Two-factor ANOVA revealed a main effect of trial ($P < 0.009$) and a significant interaction ($P < 0.039$) for body mass. On the control trial body mass was 72.7 ± 2.3 kg at the start of the trial and 72.9 ± 2.4 kg at the end of the trial. On the exercise trial body mass was 72.3 ± 2.3 kg at the start of the trial and 72.1 ± 2.3 kg at the end of the trial. Bonferroni post hoc tests revealed that body mass at the end of the exercise trial was significantly ($P < 0.003$) lower than body mass at the end of the control trial. There was a trend (Student's *t*-test, $P = 0.061$) for water consumption to be higher during the exercise trial (2168 ± 335 mL) compared with the control trial (1468 ± 114 mL).

Temperature and Humidity

There was no difference (Student's *t*-test, $P = 0.502$) in environmental temperature between the control and the exercise trials (26.2 ± 0.8 compared with 25.6 ± 1.0 °C respectively). Likewise there was no difference (Student's *t*-test, $P = 0.960$) in

humidity between the control and the exercise trials (39.4 ± 3.1 compared with $49.2 \pm 4.1\%$ respectively).

Hunger

Two-factor ANOVA revealed a main effect of time ($P < 0.0005$) and a trial \times time interaction effect ($P = 0.001$) for hunger indicating that responses differed over time between the exercise and control trials. Post hoc analysis indicated between trial differences at 08:30, 09:00 and 15:00 h but after adjusting for multiple comparisons using the Bonferroni method the only difference to remain significant ($P = 0.003$) was that at 15:00 h (**Figure 1**). Between trial differences in hunger ratings were also evaluated using AUC values for the three hours prior to the meal (08:00 to 11:00 h), the three hours after the meal (11:00 to 14:00 h), the six hours after the meal (11:00 to 17:00 h) and the full nine hours (08:00 to 17:00 h). A significant difference was found over the first three hours (08:00 to 11:00 h): 32 versus 24 (mean values) for the control and exercise trials respectively (Student's *t*-test, $P = 0.013$). The difference between AUC values for the six hours after the meal (11:00 to 17:00 h) approached significance: 44 versus 50 (mean values) for the control and exercise trials respectively (Student's *t*-test, $P = 0.056$).

INSERT FIGURE 1 NEAR HERE

Acylated ghrelin

Fasting plasma acylated ghrelin concentrations did not differ significantly between the control and exercise trials: control 150.3 ± 56.4 , exercise 137.5 ± 46.8 $\text{pg}\cdot\text{mL}^{-1}$, $P < 0.274$. Two-factor ANOVA revealed a main effect of trial ($P = 0.022$), a main

effect of time ($P = 0.048$) and a trial \times time interaction ($P = 0.043$) for acylated ghrelin concentrations. Post hoc analysis indicated between trial differences at 08:30, 09:00 and 17:00 h but after adjusting for multiple comparisons using the Bonferroni method the only difference to remain significant ($P < 0.001$) was that at 08:30 h (**Figure 2**). Area under the acylated ghrelin concentration versus time curve was 38% lower over the first three hours of the exercise trial and 35% lower over the full nine hours of the exercise trial compared with the control trial (**Figure 3**).

INSERT FIGURE 2 NEAR HERE

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Glucose and insulin

Fasting plasma glucose concentrations did not differ significantly (Student's t -test, $P = 0.613$) between trials (control 5.4 ± 0.1 mmol \cdot L $^{-1}$, exercise 5.3 ± 0.1 mmol \cdot L $^{-1}$). Two-factor ANOVA revealed a main effect of trial ($P = 0.013$), a main effect of time ($P < 0.0005$) and a trial \times time interaction ($P = 0.021$) for plasma glucose. Post hoc analysis indicated between trial differences at 08:30, 09:00 and 12:00 h but after adjusting for multiple comparisons using the Bonferroni method none of these remained significant (**Figure 4**). Total area under the concentration versus time curve for plasma glucose was higher on the exercise trial compared with the control trial over the full nine hours: 50.6 ± 1.0 compared with 47.3 ± 0.8 mmol \cdot L $^{-1}\cdot$ 9 h for the exercise and control trials respectively (Student's t -test, $P = 0.016$). The glucose AUC value was also higher for the one-hour exercise bout (08:00 to 09:00 h) in comparison with the same time period in the control trial: 6.48 ± 0.47 compared with 5.28 ± 0.09 mmol \cdot L $^{-1}\cdot$ 1 h for exercise and control respectively (Student's t -test, $P = 0.036$).

Fasting plasma insulin concentrations did not differ significantly (Student's *t*-test, $P = 0.980$) between trials (control $208.4 \pm 20.1 \text{ pmol}\cdot\text{L}^{-1}$, exercise $207.7 \pm 34.0 \text{ pmol}\cdot\text{L}^{-1}$). Two-factor ANOVA revealed a main effect of time ($P < 0.0005$) for plasma insulin but there was no main effect of trial and no interaction effect (**Figure 4**).

INSERT FIGURE 4 NEAR HERE

Correlations between acylated ghrelin and other variables.

Fasting plasma acylated ghrelin concentrations were not significantly correlated with BMI, body mass, waist circumference, maximum oxygen uptake, fasting plasma insulin concentrations or fasting plasma glucose concentrations. A significant correlation ($r = 0.699$, $P = 0.036$) was observed between AUC values for plasma acylated ghrelin and hunger over the first three hours of the exercise trial (08:00 to 11:00 h). Correlations between plasma acylated ghrelin and hunger over other periods (11:00 to 14:00, 11:00 to 17:00 and 08:00 to 17:00 h) were not significant either for the exercise trial or the control trial. When examining correlations between plasma acylated ghrelin and hunger ratings at individual time points (08:00, 08:30, 09:00, 11:00, 12:00 and 17:00 h) a significant correlation ($r = 0.781$, $P = 0.013$) was observed at 09:00 h on the exercise trial but not at any other time points on the exercise trial and not at any time points on the control trial. However, upon removal of an outlier who had very high plasma acylated ghrelin concentrations, five significant ($P < 0.05$) correlations emerged between hunger ratings and plasma acylated ghrelin concentrations. These were on the control trial at 08:00, 08:30, 09:00 and 17:00 h and on the exercise trial at 09:00 h. Correlation coefficients ranged from r

= 0.715 to $r = 0.894$. Plasma acylated ghrelin concentrations were not significantly correlated with plasma glucose or insulin concentrations at any time point during the trials.

DISCUSSION

To our knowledge, the present study is the first to examine plasma acylated ghrelin concentrations during exercise. The novel finding arising from this study is that there is a suppression of plasma acylated ghrelin during running. These findings suggest that acylated ghrelin responds differently to an exercise stimulus compared with total ghrelin, although a limitation of the study with regard to this finding is that total ghrelin concentrations were not measured. The present study also indicates that appetite is suppressed during exercise and to some extent in the immediate post-exercise period.

Although the present study is the first to examine plasma acylated ghrelin during exercise, several previous studies have investigated the response of plasma total ghrelin to exercise (7, 11, 14, 24, 25, 32, 43, 49). The findings of most of these studies indicate plasma total ghrelin concentrations are unaffected by a single session of exercise although one study has reported that serum total ghrelin concentrations are suppressed for up to an hour after the cessation of exercise (49) while another has reported that plasma total ghrelin concentrations are elevated during prolonged (three hours) moderate intensity exercise (11). We did not measure plasma total ghrelin in the present study but in a previous study using a similar exercise protocol we found no change in plasma total ghrelin concentration during or after exercise (7).

It is possible that previous studies failed to detect changes in ghrelin because they measured total ghrelin and not acylated ghrelin. Hosoda and colleagues (23) have reported that acylated ghrelin responds more quickly than total ghrelin in response to glucose ingestion and more dynamically i.e. the percentage changes in acylated

ghrelin are greater than those for total ghrelin in response to glucose ingestion. It is possible that the same applies during exercise. It is known that splanchnic blood flow is reduced during intense exercise (42) and this would reduce oxygen delivery to the stomach and intestines. It is possible that this reduced oxygen delivery interferes in some way with the secretion of ghrelin, altering the ratio of total to acylated ghrelin but we have no evidence to support this and the suggestion is highly speculative.

The finding that there is a suppression of hunger during and to some extent after exercise is consistent with results from previous studies that have monitored subjective hunger ratings following vigorous exercise (above 60% of maximum oxygen uptake) (4, 26, 27). As in previous studies the suppression of hunger was short lived and hunger ratings returned to control values within two hours of the cessation of exercise. The positive correlation between AUC values for plasma acylated ghrelin and hunger over the first three hours of the exercise trial (08:00 to 11:00 h) indicates a tendency for higher acylated ghrelin concentrations in those with higher hunger ratings. This provides support for the hypothesis that acylated ghrelin and hunger are related during and immediately after exercise.

In addition to the decline in plasma acylated ghrelin concentrations during exercise, acylated ghrelin concentrations declined after feeding in both the control and exercise trials. This is consistent with data from studies examining the response to feeding of both plasma acylated ghrelin (1, 3, 17, 23, 34) and plasma total ghrelin (3, 12, 13, 23, 34). Macronutrient composition may influence the extent to which feeding suppresses ghrelin. There is evidence to suggest that carbohydrate has a greater suppressive effect on plasma acylated ghrelin than protein or fat (17) and at least two studies have

reported that plasma acylated ghrelin concentrations are not suppressed significantly after high fat meals (1, 47). Our findings conflict with these latter findings since the meal employed in the present study was high in fat. However, this meal was also high in energy content (4200 kJ for an individual with a body mass of 70 kg) and this has been shown to impact on the suppression of total ghrelin (8). Moreover high fat meals have been shown to suppress serum total ghrelin concentrations (40). Considering the similarity in the response of acylated and total ghrelin to feeding, it is difficult to understand why there is a divergent response to exercise i.e. no change in total ghrelin but a reduction in acylated ghrelin. The findings suggest that the mechanism by which exercise alters acylated ghrelin may differ from that by which feeding alters acylated ghrelin. This is feasible because factors other than feeding, such as circadian rhythms and body composition, have been demonstrated to influence total plasma ghrelin concentration (18, 38).

The mechanisms by which feeding suppresses ghrelin concentration are thought to involve post gastric feedback. Intravenous infusion of insulin suppresses plasma total ghrelin concentrations in humans (19) as does intravenous infusion of glucose (44). In individuals with type 1 diabetes meal intake suppresses total plasma ghrelin concentrations when insulin is administered but not in the absence of insulin (35). Moreover, at least one study has shown an inverse correlation between the percentage decrease in plasma total ghrelin concentration and the percentage increase in plasma insulin and glucose concentration after meals (3). In the present study the area under the curve values for plasma glucose concentration were elevated during the one hour run compared with the same time period during the control trial. This is consistent with the findings of previous studies which also demonstrate an increase in plasma

glucose during exercise particularly in trained subjects (28, 31). This may in part explain the lower area under the curve values for plasma acylated ghrelin during the exercise trial. Plasma insulin concentrations were not elevated during exercise in the present study, in line with previous findings (28, 31), and are therefore unlikely to contribute to the suppression of plasma acylated ghrelin during exercise.

In the present study there was a tendency for higher hunger ratings over the last five hours of the exercise trial compared with the control trial. This is probably because subjects were in energy deficit during the exercise trial compared with the control trial. Previous research has demonstrated an increase in energy intake after an acute bout of exercise (39) and after a one-week period of exercise training (45). This is not a universal finding since one study has reported no increase in energy intake over a two day period following acute bouts of exercise (27). Although there was a transient reduction in appetite after exercise in the present study, it is likely that appetite was elevated later in the day due to the 3.9 MJ (930 kcal) energy expenditure resulting from the run. However, the nine hour values for plasma acylated ghrelin concentration do not support the notion that hunger was elevated at the end of the exercise trial. In fact, plasma acylated ghrelin concentrations at nine hours suggest that hunger was still suppressed on the exercise trial compared with the control trial. This apparent contradiction requires further study.

The present study had several limitations. Firstly the subjects were young and well trained and therefore the findings may not apply to older subjects or to untrained subjects. Secondly the low sample size may have limited the power to detect significant relationships between plasma acylated ghrelin and other variables. Thirdly,

plasma acylated ghrelin was measured at only six time points over the nine hour observation period. More frequent measurements of plasma acylated ghrelin would provide a clearer picture of the responses to exercise and feeding. Finally, plasma total ghrelin concentration was not measured, limiting our ability to determine the extent of variation between the responses of plasma acylated ghrelin and plasma total ghrelin to exercise and feeding.

In conclusion, this study demonstrates that plasma acylated ghrelin concentration is reduced during an acute bout of treadmill running and this lends support for the role of acylated ghrelin in appetite suppression during and immediately after exercise. Further research is required to determine the influence of other modes, durations and intensities of exercise on plasma acylated ghrelin concentration and to document plasma acylated ghrelin responses to exercise in different subject groups e.g. older subjects, untrained subjects, obese subjects. Such research could have important implications regarding the role of exercise in weight management.

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DISCLOSURES

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LEGENDS FOR FIGURES

FIGURE 1

Subjective feelings of hunger using a 0-15 scale during the exercise and control trials. Values are mean \pm SEM, $n = 9$. The shaded rectangle indicates the treadmill run. The black rectangle indicates the test meal consumption. *Significantly different from the control trial after Bonferroni adjustment $P = 0.003$.

FIGURE 2

Plasma acylated ghrelin concentration during the exercise and control trials. Values are mean \pm SEM, $n = 9$. The shaded rectangle indicates the treadmill run. The black rectangle indicates the test meal consumption. *Significantly different from the control trial after Bonferroni adjustment $P = 0.001$.

FIGURE 3

Total area under the concentration versus time curve (AUC) for plasma acylated ghrelin (mean \pm SEM, $n = 9$). Values are for the first three hours of the trial ($\text{pg}\cdot\text{mL}^{-1}\cdot 3 \text{ h}$) and for the full nine hours of the trial ($\text{pg}\cdot\text{mL}^{-1}\cdot 9 \text{ h}$). *Significantly different from the control trial, Student's t -test $P = 0.033$. †Significantly different from the control trial, Student's t -test $P = 0.021$.

FIGURE 4

Plasma glucose and insulin concentrations during the exercise and control trials. Values are mean \pm SEM, $n = 9$. The shaded rectangle indicates the treadmill run. The black rectangle indicates the test meal consumption.







