A single session of treadmill running has no effect on plasma total ghrelin concentrations

Stephen F Burns, David R Broom, Masashi Miyashita, Claire Mundy and David J Stensel (✉)

School of Sport and Exercise Sciences

Loughborough University

Loughborough

Leicestershire

LE11 3TU, UK

Correspondence and requests for reprints:

Dr David Stensel

School of Sport and Exercise Sciences

Loughborough University

Leicestershire

LE11 3TU, UK

Phone: +44 (0)1509 226344

Fax: +44 (0)1509 226301

E-mail: D.J.Stensel@lboro.ac.uk

Acknowledgements

We thank all of the subjects who participated in this study. None of the authors have any conflict of interest regarding the findings reported in this study.

A single session of treadmill running has no effect on plasma total ghrelin concentrations

Abbreviated Title: Treadmill running and plasma ghrelin

Key Words: hunger, appetite, exercise, weight control

Abstract

Ghrelin is a hormone stimulating hunger. Intense exercise has been shown to temporarily suppress hunger post-exercise. The present study investigated whether post-exercise hunger suppression is mediated by reduced plasma total ghrelin concentrations.

Nine men and nine women participated in this study. Age, body mass index and maximal oxygen uptake ($\dot{V}O_2$ max) of the participants (mean ± $s_{\bar{x}}$) were: 24.8 ± 0.9 yr, 22.9 ± 0.6 kg·m^2 and 57.7 ± 2.2 mL·kg^{-1}·min^{-1}. Participants completed two, three-hour trials (exercise and control) on separate days in a randomised balanced design after overnight fasts. The exercise trial involved a one-hour treadmill run at 73.5% of $\dot{V}O_2$ max followed by two hours of rest. The control trial involved three hours of rest. Blood samples were collected at 0, 0.5, 1, 1.5, 2 and 3 hours. Total ghrelin concentrations were determined from plasma. Hunger was assessed following blood samples using a 15-point scale. Data were analysed via repeated measures ANOVA.

Hunger scores were lower in the exercise trial compared with the control trial (Trial P=0.009; Time P<0.001; Interaction P<0.001). Plasma total ghrelin concentrations did not differ between trials.

These findings indicate that treadmill running suppresses hunger but this effect is not mediated by changes in plasma total ghrelin concentration.

Introduction

Ghrelin is a hormone that is secreted by the stomach and in smaller amounts from the hypothalamus (Kojima et al., 1999). Ghrelin concentrations rise just before meals and decrease rapidly after meals suggesting that ghrelin is involved in the acute regulation of hunger (Ariyasu et al., 2001, Cummings et al., 2001). This is supported by the finding that infusion of ghrelin leads to a short-term increase in hunger in humans (Wren et al., 2001). Plasma total ghrelin concentrations correlate negatively with body mass index (BMI) (Ikezaki et al., 2002, Soriano-Guillen et al., 2004, Tschop et al., 2001) and are responsive to diet and exercise induced changes in body mass (Cummings et al., 2002, Foster-Schubert et al., 2005, Leidy et al., 2004) indicating that ghrelin also has a role in regulating energy balance.

To our knowledge only four studies have examined the influence of an acute bout of aerobic exercise on total plasma ghrelin (Dall et al., 2002, Kallio et al., 2001, Kraemer et al., 2004a, Schmidt et al., 2004). The findings of these studies are consistent and indicate that a single session of aerobic exercise has no influence on total plasma ghrelin concentration. However, only one of these studies employed a control trial (Kraemer et al., 2004a). Moreover, in three of these studies the duration of exercise was relatively short (<30 min) and none of these studies included an assessment of hunger.

There is evidence that intense exercise (> 60% of $\dot{V}O_2$ max) causes a temporary post-exercise suppression of hunger (King et al., 1994, King and

Blundell, 1995.). This is possibly due to a decline in splanchnic blood flow during exercise (Rowell, 1974) although other mechanisms may be responsible. If it could be shown that exercise suppresses plasma total ghrelin concentration and hunger simultaneously this would: a) support previous research findings indicating that intense exercise suppresses hunger, b) indicate a mechanism by which exercise and hunger are related. Exercise may then be recommended as an alternative to pharmacological methods (currently being developed) for lowering plasma total ghrelin concentration, reducing hunger and controlling weight.

Therefore, in view of the limitations of current research we decided to re-examine the relationship between exercise and plasma total ghrelin concentration using a greater exercise stimulus (i.e. greater exercise intensity and duration and therefore greater energy deficit) than has been examined previously. We also sought to link changes in plasma total ghrelin concentration with changes in feelings of hunger – this has not been monitored in previous studies. Our primary hypothesis was that prolonged, intense exercise (1 hour at 73.5% of $\dot{V}O_2$ max) would lead to a short-term suppression of hunger which would be linked to suppressed plasma total ghrelin concentration. A secondary hypothesis was that two hours after exercise, hunger ratings and plasma total ghrelin concentrations would be higher on the exercise compared with the control trial due to the energy deficit created by the exercise.

Methods

Participants

Eighteen healthy volunteers (nine male and nine female) aged 19-32 years participated in this study, which was approved by the University's Ethical Advisory Committee. The participants gave written informed consent after receiving an explanation of the procedures and risks involved. Participants completed a health screen questionnaire and a physical activity questionnaire. Participants were recruited only if they met the following criteria: were non-smoking, were not currently on a weight gain/weight loss diet and had not been on any such diet during the previous six months, had maintained a stable weight in the previous six months, had no gastric or digestive problems, had no known history of cardiovascular disease, had resting arterial blood pressure <140/90 mm Hg.

Some physical characteristics of the participants are shown in Table 1. As a group these individuals were highly fit (mean $\dot{V}O_2$ max of 63 and 52 $mL \cdot kg^{-1} \cdot min^{-1}$ for men and women, respectively). All participants reported that they were involved in some form of regular physical activity. The most common form of activity was games sports (soccer, rugby, hockey, basketball) but some participants also performed weight training and recreational running.

TABLE 1 NEAR HERE

Preliminary tests

Anthropometry: Height was assessed using a Holtain fixed wall stadiometer (Seca, Germany). Measurements were taken to the nearest 0.1 cm. Body mass was measured using a beam balance (Avery, Birmingham, U.K.). Measurements were taken to the nearest 0.01 kg. Skinfold thickness was measured at four sites (triceps, biceps, subscapular and suprailiac) on the right hand side of the body using calipers (John Bull, U.K.). Body density was calculated using a four site formula and body fat percentage then estimated using the Siri equation (Durnin and Womersley, 1974).

Submaximal treadmill test: A 16 minute, four-stage, submaximal treadmill test was used to determine the relationship between running speed and oxygen consumption. Initial running speed was set between 8 and 9 $km \cdot h^{-1}$ depending upon participants' running ability. The treadmill was level throughout the test. Speed was increased by between 1 and 1.6 $km \cdot h^{-1}$ every 4 minutes depending on participants' fitness. Expired air samples, heart rate and ratings of perceived exertion (Borg, 1973) were collected during the final minute of each stage. A linear regression equation was used to calculate the relationship between running speed and oxygen consumption.

Maximum oxygen uptake test: $\dot{V}O_2 max$ was determined using an incremental protocol in three-minute stages (Taylor et al., 1955). Treadmill speed remained constant throughout the test. The initial incline of the treadmill was 3.5%. Treadmill gradient was increased by 2.5% every 3

minutes. Expired air samples, heart rate and ratings of perceived exertion were collected from 1:45 to 2:45 minutes of each stage and throughout the final minute of the test. Participants determined the end point of the test by indicating to the experimenters when they felt they could run for only one further minute. The final expired air collection was started at that point. Strong verbal encouragement was given to participants throughout the test. Criteria for $\dot{V}O_2$ max included two or more of the following: 1) heart rate within ± 10 $b \cdot min^{-1}$ of age-predicted maximum heart rate, 2) a respiratory exchange ratio value ≥ 1.15, 3) a plateau in oxygen consumption.

Main trials

Two main trials (exercise or control) were performed in a counterbalanced, randomised design. The interval between the two trials was at least one week. For each trial the participants reported to the laboratory at 08.00 hours after a 10-hour overnight fast. A cannula was inserted into a forearm or antecubital vein and the participants rested quietly for ten minutes. During this period participants were asked to rate their hunger (see below). In the control trial participants continued resting (reading, working quietly, watching television) for the next three hours. In the exercise trial participants performed a one-hour treadmill run (see below) and then rested for two hours.

Blood samples were obtained at baseline and at 0.5, 1, 1.5, 2 and 3 hours after baseline. The cannula was kept patent by flushing with nonheparinised saline (9 $g \cdot L^{-1}$, B.Braun Medical Ltd, Buckinghamshire, UK). The first 2 mL

of blood withdrawn was always discarded to avoid dilution of the sample. Participants were always lying in a supine position for at least five minutes before blood samples were taken except for the 0.5 and 1 hour samples taken during the exercise trial. For these samples participants straddled the treadmill while blood was being drawn. This process took approximately one minute. Water was available ad libitum during both trials and the volume ingested was recorded. Hunger was reassessed at each blood sampling point.

One-hour treadmill run

Participants were instructed that the exercise was designed to be a 'hard run' for one hour. Participants were initially set running at a speed calculated to elicit 75% of their $\dot{V}O_2$ max. If the run was too difficult for participants the speed of the treadmill was lowered. However, the speed was still maintained to produce a high intensity. Expired air samples were collected into 200 L Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) at 14-15, 29-30, 44-45 and 59-60 minutes during the run. Heart rate was measured using short-range telemetry (Polar Electro, OV), and ratings of perceived exertion were recorded during collections of expired air. Oxygen consumption and carbon dioxide production were determined from expired air samples using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex Analyser Series 1400; Servomex, Crowborough, East Sussex, U.K.). Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, U.K.) and corrected to standard temperature and pressure (dry). Energy expenditure during exercise, substrate utilisation,

carbohydrate oxidation rate ($g \cdot min^{-1}$) and fat oxidation rate ($g \cdot min^{-1}$), were calculated using equations for energy expenditure assuming no protein oxidation (Frayn, 1983).

Hunger scale

A 15-point visual scale was used to assess hunger. Participants indicated their perceived level of hunger by pointing to a number which best represented how hungry they felt. The following phrases were included on the scale: not hungry, fairly hungry, hungry and very hungry. The visual scale was validated against the visual analogue scales developed by King and colleagues (King et al., 1996, King et al., 1994). The responses were identical.

Control for diet and exercise

For two days preceding the main trials participants were asked to replicate their physical activity. Participants weighed and recorded all food and drink consumed during the 48 hours immediately preceding their first trial and they replicated this intake during the 48 hours prior to their second trial. Participants were asked to refrain from alcohol consumption during these periods. There was no control for menstrual cycle phase amongst female participants in this study.

Analytical methods

At each sampling point, blood samples were collected into pre-cooled 9mL

potassium-EDTA monovettes (Sarstedt Monovette Potassium EDTA 1.6mg EDTA/mL blood, Sarstedt, Germany) that were kept on ice until centrifugation (Koolspin Refrigerated Centrifuge, Burkard Scientific, Uxbridge, Middlesex, U.K.). Plasma was separated within 15 min of collection, divided into aliquots, and stored at -80°C.

Plasma samples were analysed for total ghrelin concentration by enzyme immunoassay (Phoenix Pharmaceuticals) using a plate reader (Opsys Microplate Reader, Dynex Technologies Inc., Franklin MA, U.S.). Glucose (Randox Laboratories Ltd. U.K.) and NEFA (Wako Chemicals GmbH, Germany) were analysed from plasma samples by enzymatic, colorimetric methods using an automated centrifugal analyser (Cobas Mira Plus; Roche, Basel, Switzerland). Plasma insulin concentration was determined using a solid-phase ^{125}I radioimmunoassay available in a commercial kit (MP Biomedicals, Orangeburg, NY, U.S.). Radioactivity was measured using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL, U.S.). Haemoglobin concentration and haematocrit were determined from blood samples collected at baseline and three hours so that changes in plasma volume could be estimated (Dill and Costill, 1974). The within batch coefficients of variation for the assays were as follows: ghrelin 9.6%, glucose, 1.3%, NEFA 0.8%, insulin 5.7%. To eliminate inter-assay variation, samples from both trials for each participant were analysed in the same batch.

Data analysis

Results were analysed using statistical software (SPSS 11.0, SPSS Inc., Chicago, IL, U.S.). Fasting and area under the curve values were compared between trials using *t*-tests for correlated data. Where gender comparisons were required independent *t*-tests were used. Repeated measures two-way ANOVA was used to determine differences between trials and over time for measurements of hunger and plasma concentrations of total ghrelin. Where appropriate post-hoc pair wise comparisons were made using the Bonferroni method. Relationships between variables were evaluated using Pearson's product-moment correlation coefficient. A 5% level of significance was adopted throughout, and data are expressed as mean $\pm$ $s_{\overline{x}}$.

Results

Responses to treadmill running

Average heart rate during exercise was 173 ± 2 b·min^{-1}. This represented 91 ± 1% of maximum heart rate. The mean % $\dot{V}O_2$ max elicited during exercise was 73.5 ± 0.8% and the mean respiratory exchange ratio was 0.89 ± 0.01. Gross energy expenditure during exercise was 3747 ± 207 kJ with 35 ± 3% of energy provided from fat and 64 ± 3% of energy provided from carbohydrate. The median rating of perceived exertion during exercise was 15 i.e. 'hard' (range 13-16).

Fluid consumption and body mass

Participants consumed more water (P<0.001) during the exercise trial (978 ± 115 mL) compared to the control trial (443 ± 76 mL). Body mass did not differ between trials at baseline. Body mass was lower (P=0.006) at the end of the exercise trial (i.e. at 3 hours) compared with the end of the control trial (67.9 ± 2.6 kg *versus* 68.5 ± 2.6 kg for exercise and control respectively).

Hunger

Hunger scores (Figure 1) were suppressed during and after exercise: main effect of trial (P=0.009), main effect of time (P<0.001), trial × time interaction (P<0.001). Post-hoc tests revealed that hunger scores were lower during the exercise *versus* control trial at 0.5, 1, 1.5 and 2 hours (all P<0.05). There was a main effect of time and a trial × time interaction for both sexes

for hunger. However a main effect of trial was not found for either sex in isolation. Males: trial P=0.059, time P<0.001, trial × time interaction P=0.022; females: trial P=0.100, time P<0.001, trial × time interaction P=0.004.

FIGURE 1 NEAR HERE

Hormone and substrate concentrations at baseline

Baseline plasma concentrations are shown in Tables 2 and 3. There were no differences between the control and exercise trials for any of the hormones/metabolites at baseline. Although baseline plasma total ghrelin concentrations tended to be higher for the males than the females on both the control and exercise trials these differences were not significant (P=0.52 and P=0.54 for the control and exercise trials respectively).

Hormone and substrate responses to exercise

Changes in plasma volume over the period of observation were small and did not differ (P=0.865) between control (-0.6 ± 1.7%) and exercise (0.0 ± 3.3%) trials. Therefore, no adjustments were made to measured concentrations of plasma constituents.

There was no significant difference in plasma total ghrelin concentrations between trials or over time in either the group as a whole (Figure 2) or the males or females separately. Area under the curve values for plasma total

ghrelin concentration did not differ significantly between the exercise and control trials for the males, the females or the group as a whole (Table 2). Although the area under the curve values tended to be higher for males than females on both the control and the exercise trials these gender differences were not significant (*P*=0.457 for the control trial and *P*=0.302 for the exercise trial, *t*-tests for correlated data).

TABLE 2 NEAR HERE

FIGURE 2 NEAR HERE

Area under the curve values for insulin, glucose and NEFA are shown in Table 3. Area under the curve values for NEFA and glucose were higher on the exercise than the control trial for the group as a whole (*P*=0.007 for NEFA, *P*=0.004 for glucose).

TABLE 3 NEAR HERE

Mean fasting plasma total ghrelin concentrations (i.e. control trial concentration plus exercise trial concentration divided by two) were not significantly correlated with BMI, body mass, body fat percentage, waist circumference, insulin, glucose or $\dot{V}O_2$ max for the group as a whole. For the males a negative correlation between fasting plasma total ghrelin concentration and BMI was observed (*r*=-0.726, *P*=0.027) and both body fat percentage (*r*=-0.626, *P*=0.071) and waist circumference (*r*=-0.606, *P*=0.084)

297 showed a trend toward significant negative correlations with plasma total
298 ghrelin concentration. No significant correlations were observed between
299 fasting plasma total ghrelin concentration and any of the above variables for
300 the females.

Discussion

The main finding in the present study is that hunger was suppressed during and after treadmill running whereas plasma total ghrelin concentration was unaffected. The lack of change in plasma total ghrelin concentration during aerobic exercise is consistent with the findings of previous studies (Dall et al., 2002, Kallio et al., 2001, Kraemer et al., 2004a, Schmidt et al., 2004). However, the present study extends the findings of these studies by showing that plasma total ghrelin concentrations are unrelated to feelings of hunger during and following exercise, which has not been examined previously.

The volume of exercise performed in the present study would have induced a greater energy deficit compared to that in previous studies (Dall et al., 2002, Kallio et al., 2001, Kraemer et al., 2004a, Kraemer et al., 2004b, Schmidt et al., 2004). We employed a high volume and intensity of exercise for two reasons. Firstly, we attempted to provoke a temporary suppression of hunger which we thought might be linked to suppressed concentrations of plasma total ghrelin. Secondly, we hypothesised that the large energy deficit (3747 kJ = approximately 900 kcal) would result in an elevated plasma total ghrelin concentration two hours post exercise when feelings of hunger had returned and possibly increased. Support for this notion comes from the finding that plasma total ghrelin concentration is elevated in women who are in a state of chronic energy deficit as evidenced by amenorrhoea or anorexia (De Souza et al. 2004, Otto et al. 2001). In the present study, the elevated NEFA concentrations on the exercise trial suggest that participants were in an acute

state of negative energy balance compared with the control trial. However, there was no evidence that plasma total ghrelin concentrations were increased at any point in the exercise trial.

The suppressed hunger ratings observed in the present study lasted for at least one hour post-exercise. There was no difference in hunger at the start or end of the trials in the present study, thus the suppression in hunger seen here suggests a temporary exercise-induced anorexia (King et al., 1994, King and Blundell, 1995). It is known that during exercise there is redistribution of blood flow away from the splanchnic circulation towards the working muscles (Rowell, 1974). Since ghrelin is produced in the stomach (Kojima et al., 1999) and blood flow to this region is reduced during exercise we speculated that ghrelin concentrations would also be reduced. Another reason for expecting exercise induced suppression of ghrelin is that exercise increases growth hormone secretion (Schmidt et al 2004) and this is thought to down regulate ghrelin secretion (Korbonits et al 2004). However, ghrelin may stimulate changes in hunger via afferent activity of the vagus nerve (Hosoda et al. 2002). Therefore, it is possible that exercise could influence hunger by altering ghrelin signalling through the vagus nerve without changing circulating ghrelin concentrations.

Plasma ghrelin concentrations have been shown to change in response to individual meals (Ariyasu et al., 2001, Cummings et al., 2001), although this is not a universal finding (English et al., 2002) and at least one study has

demonstrated a preservation of meal related ghrelin responses in subjects who fasted for 24 hours (Natalucci et al. 2005). The acute change in ghrelin following food intake was one factor that led us to hypothesize that plasma total ghrelin concentration might respond acutely to exercise. However, food intake could influence ghrelin concentrations via mechanisms that are less applicable to exercise.

The presence of nutrients in the gut (Caixas et al., 2002) and increases in insulin (Flanagan et al., 2003) and glucose (Nakagawa et al., 2002) concentrations in the blood have all been associated with reductions in plasma total ghrelin concentration. Such changes do not necessarily occur during or following an acute bout of exercise. Plasma insulin concentrations, for example, were unaffected by exercise in the present study although plasma glucose concentrations were elevated. Moreover, short-term (4-day) energy restriction (-3360 kJ/d) has been found to have no effect on fasting and postprandial plasma total ghrelin concentrations (Doucet et al., 2004). Therefore, perhaps plasma total ghrelin concentrations are more sensitive to acute changes in nutrient intake than to acute physiological changes (redistribution of blood flow, short-term energy deficit) induced by exercise.

Some studies have reported that plasma total ghrelin concentrations are negatively correlated with BMI, body fat percentage and waist circumference (Ikezaki et al., 2002, Tschop et al., 2001). In the present study BMI was negatively correlated with plasma total ghrelin concentration in the male

group. Moreover, body fat percentage and waist circumference showed a trend towards a significant negative correlation with plasma total ghrelin concentration in the males. Possibly the range of values was too narrow in the present study to produce statistically significant correlations. However, the trends in the present study for males support previous evidence that plasma total ghrelin concentration is related to body composition.

The present study did not control for menstrual cycle phase between trials for female participants. No study has systematically investigated plasma total ghrelin concentration changes over the course of the menstrual cycle. However, Barkan and colleagues (2003) reported that plasma total ghrelin concentration (measured in the late follicular stage of the menstrual cycle) was higher in five young women compared to six young men. Conversely, Tschop and colleagues found no sex differences for plasma total ghrelin concentration in either Caucasians or Pima Indians (Tschop et al., 2001). Similarly, Purnell and co-workers reported that fasting plasma total ghrelin concentrations did not differ in 21 male and 39 female healthy subjects (Purnell et al., 2003). Our findings are consistent with these studies in indicating that plasma total ghrelin concentrations do not differ significantly between men and women.

Although the findings of the present study concur with the evidence currently available regarding exercise and plasma total ghrelin concentration, caution is required when interpreting the results. Ghrelin is also released in small

amounts within the central nervous system and acts directly on the hypothalamus (Kojima et al., 1999). This was not measured in the present study and it is possible that ghrelin release within the central nervous system differed between the control and exercise trials. Furthermore, ghrelin circulates in both active and inactive forms in the plasma (Kojima et al., 1999). The present study measured total plasma ghrelin concentrations (i.e. active and inactive combined) and not active ghrelin. Active ghrelin is more sensitive to changes in energy intake than total ghrelin (Hosoda et al., 2004) and it is possible that active ghrelin may respond to exercise. Nevertheless, previous studies have demonstrated changes in plasma total ghrelin concentration in response to meals (Ariyasu et al., 2001, Cummings et al., 2001) suggesting that changes in total ghrelin do reflect changes in active ghrelin in some situations.

In conclusion our findings indicate that a one-hour bout of high intensity treadmill running leads to a temporary suppression of hunger. However, this effect does not appear to be mediated through a decrease in plasma total ghrelin concentration. This suggests that plasma total ghrelin concentration is not responsive to acute exercise induced alterations in metabolism.

References

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K. and Nakao, K. (2001). Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *Journal of Clinical Endocrinology & Metabolism,* 86, 4753-4758.

Barkan, A. L., Dimaraki, E. V., Jessup, S. K., Symons, K. V., Ermolenko, M. and Jaffe, C. A. (2003). Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. *Journal of Clinical Endocrinology & Metabolism,* 88, 2180-2184.

Borg, G. A. (1973). Perceived exertion: a note on "history" and methods. *Medicine and Science in Sports,* 5, 90-93.

Caixas, A., Bashore, C., Nash, W., Pi-Sunyer, F. and Laferrere, B. (2002). Insulin, unlike food intake, does not suppress ghrelin in human subjects. *The Journal of Clinical Endocrinology & Metabolism*, 87, 1902-1906.

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E. and Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes,* 50, 1714-1719.

Cummings, D. E., Weigle, D. S., Frayo, R. S., Breen, P. A., Ma, M. K., Dellinger, E. P. and Purnell, J. Q. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England Journal of Medicine*, 346, 1623-1630.

Dall, R., Kanaley, J., Hansen, T. K., Moller, N., Christiansen, J. S., Hosoda, H., Kangawa, K. and Jorgensen, J. O. (2002). Plasma ghrelin levels during exercise in healthy subjects and in growth hormone-deficient patients. *European Journal of Endocrinology*, 147, 65-70.

De Souza, M.J., Leidy, H.J., O'Donnell, E., Lasley, B. and Williams, N.I. (2004). Fasting ghrelin levels in physically active women: relationship with menstrual disturbances and metabolic hormones. *Journal of Clinical Endocrinology and Metabolism*, 89, 3536-3542.

Dill, D. B. and Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37, 247-248.

Doucet, E., Pomerleau, M. and Harper, M. E. (2004). Fasting and postprandial total ghrelin remain unchanged after short-term energy restriction. *Journal of Clinical Endocrinology & Metabolism*, 89, 1727-1732.

Durnin, J. V. G. A. and Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition,* 32, 77-97.

English, P. J., Ghatei, M. A., Malik, I. A., Bloom, S. R. and Wilding, J. P. (2002). Food fails to suppress ghrelin levels in obese humans. *Journal of Clinincal Endocrinology and Metabolism,* 87, 2984-2987.

Flanagan, D. E., Evans, M. L., Monsod, T. P., Rife, F., Heptulla, R. A., Tamborlane, W. V. and Sherwin, R. S. (2003). The influence of insulin on circulating ghrelin. *American Journal of Physiology Endocrinology and Metabolism,* 284, E313-316.

Foster-Schubert, K. E., McTiernan, A., Frayo, R. S., Schwartz, R. S., Rajan, K. B., Yasui, Y., Tworoger, S. S. and Cummings, D. E. (2005). Human plasma ghrelin levels increase during a one-year exercise program. *Journal of Clinical Endocrinology and Metabolism,* 90, 820-825.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology,* 55, 628-634.

Hosoda, H., Doi, K., Nagaya, N., Okumura, H., Nakagawa, E., Enomoto, M., Ono, F. and Kangawa, K. (2004). Optimum collection and storage conditions

for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clinical Chemistry,* 50, 1077-1080.

Hosoda, H., Kojima, M. and Kangawa, K. (2002). Ghrelin and the regulation of food intake and energy balance. *Molecular Interventions*, 2, 494-503.

Ikezaki, A., Hosoda, H., Ito, K., Iwama, S., Miura, N., Matsuoka, H., Kondo, C., Kojima, M., Kangawa, K. and Sugihara, S. (2002). Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. *Diabetes,* 51, 3408-3411.

Kallio, J., Pesonen, U., Karvonen, M. K., Kojima, M., Hosoda, H., Kangawa, K. and Koulu, M. (2001). Enhanced exercise-induced GH secretion in subjects with Pro7 substitution in the prepro-NPY. *Journal of Clinical Endocrinology and Metabolism,* 86, 5348-5352.

King, N. A. and Blundell, J. E. (1995). High-fat foods overcome the energy expenditure induced by high-intensity cycling or running. *European Journal of Clinical Nutrition,* 49, 114-123.

King, N. A., Burley, V. J. and Blundell, J. E. (1994). Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition,* 48, 715-724.

King, N. A., Snell, L., Smith, R. D. and Blundell, J. E. (1996). Effects of short-term exercise on appetite responses in unrestrained females. *European Journal of Clinical Nutrition*, 50, 663-667.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. and Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402, 656-660.

Korbonits, M., Goldstone, A.P., Gueorguiev, M. and Grossman, A.B. (2004). Ghrelin – a hormone with multiple functions. *Frontiers in Neuroendocrinology*, 25, 27-68.

Kraemer, R. R., Durand, R. J., Acevedo, E. O., Johnson, L. G., Kraemer, G. R., Hebert, E. P. and Castracane, V. D. (2004a). Rigorous running increases growth hormone and insulin-like growth factor-I without altering ghrelin. *Experimental Biology and Medicine (Maywood)*, 229, 240-246.

Kraemer, R. R., Durand, R. J., Hollander, D. B., Tryniecki, J. L., Hebert, E. P. and Castracane, V. D. (2004b). Ghrelin and other glucoregulatory hormone responses to eccentric and concentric muscle contractions. *Endocrine*, 24, 93-98.

Leidy, H. J., Gardner, J. K., Frye, B. R., Snook, M. L., Schuchert, M. K., Richard, E. L. and Williams, N. I. (2004). Circulating ghrelin is sensitive to

changes in body weight during a diet and exercise program in normal-weight young women. *Journal of Clinical Endocrinology and Metabolism*, 89, 2659-2664.

Nakagawa, E., Nagaya, N., Okumura, H., Enomoto, M., Oya, H., Ono, F., Hosoda, H., Kojima, M. and Kangawa, K. (2002). Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: responses to the intravenous and oral administration of glucose. *Clinical Science (London)*, 103, 325-328.

Natalucci G, Riedl S, Gleiss A, Zidek T, Frisch H. (2005). Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern. *European Journal of Endocrinology*, 152, 845-500.

Otto, B., Cuntz, U., Fruehauf, E., Wawarta, R., Folwaczny, C., Riepl, R.L., Heiman, M.L., Lehnert, P., Fichter, M. and Tschöp, M. (2001). Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *European Journal of Clinical Endocrinology*, 145, R5-R9.

Purnell, J. Q., Weigle, D. S., Breen, P. and Cummings, D. E. (2003). Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. *Journal of Clinical Endocrinology and Metabolism*, 88, 5747-5752.

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiological Reviews,* 54, 75-159.

Schmidt, A., Maier, C., Schaller, G., Nowotny, P., Bayerle-Eder, M., Buranyi, B., Luger, A. and Wolzt, M. (2004). Acute exercise has no effect on ghrelin plasma concentrations. *Hormone and Metabolic Research,* 36, 174-177.

Soriano-Guillen, L., Barrios, V., Campos-Barros, A. and Argente, J. (2004). Ghrelin levels in obesity and anorexia nervosa: effect of weight reduction or recuperation. *The Journal of Pediatrics,* 144, 36-42.

Taylor, H. L., Buskirk, E. and Henschel, A. (1955). Maximal oxygen intake as an objective measure of cardio-respiratory performance. *Journal of Applied Physiology,* 8, 73-80.

Tschop, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E. and Heiman, M. L. (2001). Circulating ghrelin levels are decreased in human obesity. *Diabetes,* 50, 707-709.

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., Dhillo, W. S., Ghatei, M. A. and Bloom, S. R. (2001). Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and Metabolism,* 86, 5992-5995.

584 **Figure Captions**

585

586 **Figure 1.** Subjective feelings of hunger in the fasted state over 3 hours
587 during exercise and control trials. Values are mean ± $s_{\bar{x}}$, *n*=18. Main effect
588 of trial (*P*=0.009), main effect of time (*P*<0.001), trial × time interaction
589 (*P*<0.001). *Significantly different (*P*<0.05) between trials using a
590 Bonferroni post hoc test.

591

592 **Figure 2.** Plasma total ghrelin concentrations in the fasted state over 3 hours
593 during exercise and control trials. No significant main effects. No significant
594 interaction. Values are mean ± $s_{\bar{x}}$, *n*=18.

Table 1. Physical characteristics of the subjects.

	Males (*n*=9)	Females (*n*=9)	*P*
Age (yrs)	24.5 ± 1.3	25.1 ± 1.2	0.737
Height (m)	1.78 ± 0.02	1.68 ± 0.02	0.007
Body mass (kg)	74.03 ± 4.20	63.57 ± 2.55	0.049
BMI ($kg \cdot m^2$)	23.4 ± 1.0	22.5 ± 0.8	0.501
Waist circumference (cm)	79 ± 3	76 ± 1	0.324
Body fat (%)	16.9 ± 1.7	28.3 ± 1.2	0.001
$\dot{V}O_2$ max ($mL \cdot kg^{-1} \cdot min^{-1}$)	63.2 ± 2.5	52.1 ± 2.4	0.006

Values are mean ± $s_{\bar{X}}$. Means were compared using independent *t*-tests.

Table 2. Baseline and three-hour areas under the plasma total ghrelin concentration *versus* time curve (AUC) during the control and exercise trials.

	Control	Exercise	*P*
Baseline Ghrelin			
Whole Group (pmol·L^{-1})	412.2 ± 75.6	410.2 ± 66.8	0.910
Males (pmol·L^{-1})	463.1 ± 144.0	453.1 ± 130.6	0.664
Females (pmol·L^{-1})	361.4 ± 54.1	367.3 ± 38.1	0.840
Ghrelin 3-hour AUC			
Whole Group (pmol·L^{-1}·3 h)	1374.9 ± 231.7	1240.7 ± 179.8	0.189
Males (pmol·L^{-1}·3 h)	1556.1 ± 440.6	1431.9 ± 326.5	0.383
Females (pmol·L^{-1}·3 h)	1193.7 ± 160.5	1049.5 ± 147.2	0.366

Values are mean ± $s_{\bar{X}}$. Whole Group *n*=18; Males *n*=9; Females *n*=9. Means were compared using *t*-tests for correlated data.

Table 3. Baseline and three-hour areas under the plasma concentration *versus* time curve (AUC) for insulin, NEFA and glucose during the control and exercise trials.

	Control	Exercise	*P*
Baseline			
Insulin ($pmol \cdot L^{-1}$)	158.8 ± 12.0	168.9 ± 12.5	0.455
NEFA ($mmol \cdot L^{-1}$)	0.51 ± 0.05	0.53 ± 0.06	0.799
Glucose ($mmol \cdot L^{-1}$)	5.27 ± 0.16	5.49 ± 0.18	0.273
3-hour AUC			
Insulin ($pmol \cdot L^{-1} \cdot 3$ h)	494.0 ± 33.7	492.6 ± 35.0	0.962
NEFA ($mmol \cdot L^{-1} \cdot 3$ h)	1.67 ± 0.17	2.29 ± 0.22	0.007
Glucose ($mmol \cdot L^{-1} \cdot 3$ h)	15.66 ± 0.25	16.67 ± 0.35	0.004

Values are mean ± $s_{\bar{X}}$, n=18. Means were compared using *t*-tests for correlated data. NEFA: non-esterified fatty acids.