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ABSTRACT

Acute exercise transiently suppresses the orexigenic gut hormone acylated ghrelin, but the extent exercise intensity and duration determine this response is not fully understood. The effects of manipulating exercise intensity and duration on acylated ghrelin concentrations and hunger were examined in two experiments. In experiment one, nine healthy males completed three, 4-hour conditions (control, moderate-intensity running (MOD) and vigorous-intensity running (VIG)), with an energy expenditure of ~2.5 MJ induced in both MOD (55 min running at 52% peak oxygen uptake ($\dot{V}O_{2peak}$)) and VIG (36 min running at 75% $\dot{V}O_{2peak}$). In experiment two, nine healthy males completed three, 9-hour conditions (control, 45 min running (EX45) and 90 min running (EX90)). Exercise was performed at 70% $\dot{V}O_{2peak}$. In both experiments, participants consumed standardised meals, and acylated ghrelin concentrations and hunger were quantified at predetermined intervals. In experiment one, delta acylated ghrelin concentrations were lower than control in MOD (ES=0.44, P=0.01) and VIG (ES=0.98, P<0.001); VIG was lower than MOD (ES=0.54, P=0.003). Hunger ratings were similar across the conditions (P=0.35). In experiment two, delta acylated ghrelin concentrations were lower than control in EX45 (ES=0.77, P<0.001) and EX90 (ES=0.68, P<0.001); EX45 and EX90 were similar (ES=0.09, P=0.55). Hunger ratings were lower than control in EX45 (ES=0.20, P=0.01) and EX90 (ES=0.27, P=0.001); EX45 and EX90 were similar (ES=0.07, P=0.34). Hunger and delta acylated ghrelin concentrations remained suppressed at 1.5h in EX90 but not EX45. In conclusion, exercise intensity, and to a lesser extent duration, are determinants of the acylated ghrelin response to acute exercise.

INTRODUCTION

Obesity is characterised by a chronic energy imbalance reflecting a surplus of energy intake above expenditure, and remains a major global public health and economic burden (Wang *et al*. 2011; Ng *et al.* 2014). Recent years have witnessed significant research into the relationship between exercise, appetite regulation and energy balance (Schubert *et al.* 2014). Exercise is recommended as a therapeutic weight management strategy because it increases energy expenditure which contributes to a negative energy balance if unaccompanied by an increase in energy intake (Donnelly *et al.* 2009). Evidence suggests acute exercise transiently suppresses feelings of hunger during and shortly after exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a), which has been termed 'exercise-induced anorexia' (King *et al.* 1994). Furthermore, these responses often coincide with exercise-induced fluctuations in hormones that regulate energy balance and appetite (Schubert *et al.* 2014).

Appetite and energy intake are regulated by the neuroendocrine system, of which gut peptides play an integral role as episodic signals for hunger and satiety (Karra & Batterham 2010; Hussain & Bloom 2013). Ghrelin is the only known orexigenic gut peptide, and is predominantly secreted from the stomach (Karra & Batterham 2010). Ghrelin exists in two forms – acylated and unacylated – and, although only 10–20% of circulating ghrelin is acylated, it is believed that this form is solely responsible for appetite stimulation (Ghigo *et al.* 2005). Considering the central role of acylated ghrelin in appetite regulation, it is unsurprising that the interaction between exercise and acylated ghrelin continues to attract scientific enquiry.

Acute moderate- to high-intensity exercise suppresses acylated ghrelin concentrations (King *et al.* 2013; Schubert *et al.* 2014). This hormonal alteration appears transient and typically coincides with a reduction in hunger during and immediately after exercise (Broom *et al.*

2007, 2009; King *et al.* 2010a). Exercise intensity has been identified as a potential determinant modulating the acylated ghrelin response to exercise (Broom *et al.* 2007; King *et al.* 2010a), with suppression occurring after exercise at higher (≥60% peak oxygen uptake ($\dot{V}O_{2peak}$)) (Broom *et al*. 2007, 2009; King *et al.* 2010a) but not lower (≤50% $\dot{V}O_{2peak}$) (Ueda *et al.* 2009; King *et al.* 2010b) intensities. Studies comparing acute moderate- vs. high-intensity exercise suggest exercising at a higher intensity may be more potent for suppressing acylated ghrelin concentrations (Deighton *et al.* 2013; Metcalfe *et al.* 2015). However, the effect of isoenergetic exercise bouts at different intensities has revealed contrasting findings (Sim *et al.* 2014; Martins *et al.* 2015; Howe *et al.* 2016); therefore, further research is required to elucidate the importance of exercise intensity on appetite regulation.

Alterations in ghrelin concentrations and hunger perceptions may also be influenced by manipulations in exercise duration. Erdmann *et al.* (2007) reported that 30, 60 and 120 min of cycling at 50 W resulted in a similar increase in total ghrelin concentrations (50 to 70 $pg \cdot mL^{-1}$) during exercise without any changes in hunger. However, the assessment of total ghrelin may obscure important changes in acylated ghrelin (Hosoda *et al.* 2004), and exercise studies measuring total ghrelin have yielded equivocal findings (King *et al.* 2013). The effect of exercise duration on acylated ghrelin concentrations has not yet been examined and may have important implications regarding the use of exercise as a weight control strategy.

This investigation comprises two experiments which aimed to advance understanding of appetite and hormonal responses to different acute exercise manipulations. Experiment one compared the effect of acute isoenergetic moderate- and vigorous-intensity running on acylated ghrelin concentrations and hunger perceptions. In experiment two, the acylated ghrelin and hunger responses to single bouts of 45 and 90 min running were examined.

METHODS

Participants

This investigation contains two experimental studies that were approved by the University Ethical Advisory Committee. Two different groups of healthy, recreationally active men provided their written informed consent to participate in one of the experiments. Information from a health screen questionnaire revealed that all participants were metabolically healthy, non-smokers, not taking medication, body mass stable for at least 6 months (±2 kg) and not currently dieting. Physical and physiological characteristics of participants are presented in Table 1.

Preliminary measures

Participants attended the laboratory for two preliminary visits before the main conditions in each experiment. During the first visit, anthropometric data (stature, body mass, waist circumference, skinfold thickness) were collected and participants were familiarised with exercising on the treadmill (RUNRACE, Techno gym, Gambettola, Italy).

During the second visit, participants completed two exercise tests. The first test consisted of a 16-min submaximal incremental running test to determine the relationship between running speed and oxygen consumption. Participants completed 4×4 min stages with the initial running speed set between 7–8 $km \cdot h^{-1}$ depending on the participant's fitness level, which was increased by 1–1.5 $km \cdot h^{-1}$ at the start of each subsequent stage. Oxygen consumption and carbon dioxide production were determined from expired air samples collected in the final minute of each stage along with the participant's rating of perceived exertion (RPE) using Borg's 6–20 scale (Borg 1973). Heart rate was monitored continuously using short-range telemetry (Polar A3, Kempele, Finland).

After 30-min standardised rest, $\dot{V}O_{2peak}$ was measured using an incremental uphill treadmill protocol at a constant speed (Taylor *et al.* 1955). The initial treadmill gradient was set at 3.5% which was increased by 2.5% every 3 min until volitional exhaustion (Taylor *et al.* 1955). Peak oxygen consumption was determined from an expired air sample collected during the final minute of the test when participants indicated that they could only continue for an additional 1 min. Heart rate and RPE were monitored throughout the test as described previously. Data from the two preliminary exercise tests were used to determine the running speeds required during the main conditions.

Experimental design

In each experiment, participants completed three, 1-day conditions in a random order separated by at least one week. Participants weighed, recorded and replicated their food intake in the 24-h before each main condition. Participants abstained from caffeine, alcohol and strenuous physical activity during the same period. All conditions commenced between 08:00 and 09:00 after an overnight fast of at least 10-h. The study design in both experiments is presented in Figure 1.

Experiment one: exercise intensity

Nine men (20–25 years) completed three, 4-h experimental conditions: control, moderate-intensity running (MOD) and vigorous-intensity running (VIG). Participants rested in the laboratory throughout the control condition. The exercise conditions commenced with participants running on the treadmill at a speed predicted to elicit either 50% $\dot{V}O_{2peak}$ (MOD) or 75% $\dot{V}O_{2peak}$ (VIG), which was designed to induce a gross energy expenditure of 2510 kJ. Expired air samples were collected at regular intervals to calculate the relative exercise intensity and the treadmill speed was adjusted occasionally to ensure the target intensity was met. The exercise energy expenditure and substrate oxidation were estimated via indirect

calorimetry (Frayn 1983). Heart rate was monitored throughout and RPE was recorded during the last 10 s of each expired air sampling period. After the exercise bout, participants rested in the laboratory for the remainder of the condition.

A standardised meal prescribed relative to body mass was provided at 3-h and consumed within 15 min, which consisted of white bread, tuna, mayonnaise, chocolate bar, potato crisps, apple and orange juice. The standardised meal provided 60 kJ energy, 2.13 g (56% of meal total energy) carbohydrate, 0.53 g (15%) protein and 0.47 g (29%) fat per kilogram body mass. Water was provided *ad libitum* throughout each condition.

Experiment two: exercise duration

Nine men (21–28 years) completed three, 9-h experimental conditions: control, 45 min running (EX45) and 90 min running (EX90). Participants rested in the laboratory throughout the control condition. During the exercise conditions, participants ran on the treadmill at a speed predicted to elicit 70% $\dot{V}O_{2peak}$ for 45 min (EX45) or 90 min (EX90). Expired air samples were collected at regular intervals to calculate the relative exercise intensity and the treadmill speed was adjusted occasionally to ensure the target intensity was achieved. The exercise energy expenditure and substrate oxidation were estimated via indirect calorimetry (Frayn 1983). Heart rate was monitored throughout and RPE was recorded during the last 10 s of each expired air sampling period. After the exercise bout, participants rested in the laboratory for the remainder of the condition.

Participants consumed identical standardised meals prescribed relative to body mass within 15 min at 2 and 6 h. The meals consisted of white bread, Cheddar cheese, mayonnaise, butter, potato crisps, milkshake powder and whole milk. The standardised meals provided 46 kJ energy, 0.95 g (33%) carbohydrate, 0.31 g (11%) protein and 0.69 g (56%) fat per kilogram body mass. Water was provided *ad libitum* throughout each condition.

Hunger perceptions

Ratings of perceived hunger were assessed at baseline (fasted) and every 30 min during both experiments using a 100 mm visual analogue scale (Flint *et al.* 2000). An additional measurement was taken at 45 min in experiment two.

Blood sampling

Venous blood samples were collected via a cannula (Venflon, Becton Dickinson, Helinsborg, Sweden) inserted into an antecubital vein. All samples were collected in the semi-supine position, except the samples scheduled during exercise, which were taken while participants straddled the treadmill. Plasma acylated ghrelin concentrations were determined from blood samples collected into pre-chilled 4.9 mL EDTA monovettes (Sarsedt, Leicester, UK) at 0 (baseline), 0.08, 0.5, 1, 3, 3.5 and 4 h in experiment one and at 0 (baseline), 0.75, 1.5, 2, 3, 6, 7 and 9 h in experiment two. These monovettes contained p-hydroxymercuribenzoic acid (PHMB) to prevent the degradation of acylated ghrelin by protease. Monovettes were spun at 1,287×g for 10 mins at 4°C (Burkard, Hertfordshire, UK). The plasma supernatant was aliquoted into a storage tube and 100 µL of 1 M hydrochloric acid was added per millilitre of plasma (Hosoda *et al.* 2004). Samples were re-centrifuged at 1,287×g for 5 mins at 4°C prior to storage at -80°C for later analysis.

Plasma glucose and insulin concentrations were determined from blood samples collected into pre-chilled 9 mL EDTA monovettes (Sarsedt, Leicester, UK). Glucose concentrations were measured at 0 (baseline), 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h in experiment one and at 0 (baseline), 0.75, 1.5, 2, 2.5, 3, 4, 5, 6, 6.5, 7, 8 and 9 h in experiment 2. Insulin concentrations were measured at 0 (baseline), 0.5, 1, 2, 3, 3.5 and 4 h in experiment one and at 0 (baseline), 0.75, 1.5, 2, 3, 6, 7 and 9 h in experiment two. Monovettes were centrifuged immediately at

1,681×g for 10 mins at 4°C (Burkard, Hertfordshire, UK). The plasma supernatant was aliquoted into Eppendorf tubes prior to storage at -80°C for subsequent analysis.

At each blood sampling point, haemoglobin concentration (via the cyanmethaemoglobin method) and haematocrit (via microcentrifugation) were determined to estimate acute changes in plasma volume (Dill & Costill 1974).

Biochemical analysis

In both experiments, plasma acylated ghrelin concentrations were determined using a commercially available enzyme immunoassay (SPI BIO, Montigny le Bretonneaux, France). Plasma glucose concentrations were determined using an automated centrifugal analyser (Cobas Mira Plus, Roche, Basel, Switzerland). For experiment one, plasma insulin concentrations were determined by a solid phase ^{125}I radioimmunoassay available in a commercial kit (MP Biomedicals, Orangeburg, NY) using an automated gamma counter system (Cobra II, Packard Instrument, Downers Grove, IL). For experiment two, plasma insulin concentrations were quantified using a commercially available enzyme-linked immunoassay (Mercodia, Uppsala, Sweden). The within-batch coefficient of variation for acylated ghrelin, glucose and insulin were 7.0%, 1.4% and 8.9%, respectively in experiment one and 2.2%, 0.6% and 4.7%, respectively in experiment two.

Statistical analyses

Data were analysed using IBM Statistics Software for Windows version 21 (IBM Corporation, New York, USA). Time-averaged area under the curve (AUC) values were calculated using the trapezoidal rule. Normality of the data was checked using Shapiro-Wilk tests. Normally distributed data are presented as mean (SD). Data for hunger, glucose and insulin were natural log transformed prior to analysis. These data are presented as geometric mean (95%

confidence interval) and analysis is based on ratios of the geometric means. Acylated ghrelin concentrations are presented relative to baseline concentrations (i.e., delta) to minimise the potential influence of day-to-day biological variation in this appetite hormone (Deighton *et al*. 2013).

In both experiments, linear mixed models repeated for condition were used to examine differences in exercise responses, fasting (baseline) concentrations and AUC values. Differences in metabolite concentrations between conditions over time were examined using linear mixed models repeated for condition and time. In experiment two, temporal changes in AUC responses for hunger and acylated ghrelin between experimental conditions were examined over sub-sections of the 9 h measurement period (0–2 h, 2–6 h, 6–9 h) using separate linear mixed models with condition as the sole factor. All linear mixed models included a random effect for each participant. Where significant condition and interaction effects were found, post-hoc analysis was performed using the Holm-Bonferroni correction for multiple comparisons (Atkinson 2002). Correction of acylated ghrelin, glucose and insulin concentrations for changes in plasma volume did not alter the interpretation of the results; therefore, the unadjusted values are presented for simplicity. Pearson's product moment correlations were used to examine relationships between variables. Statistical significance was accepted as $P<0.05$. Absolute standardised effect sizes (ES) are included to supplement important findings. An ES of 0.2 was considered the minimum important difference in all outcome measures, 0.5 moderate and 0.8 large (Cohen 1988). Graphical representations of results are presented as mean (SEM) to avoid distortion of the figures.

RESULTS

Experiment one: exercise intensity

Exercise responses

Exercise responses for MOD and VIG are shown in Table 2. Exercise duration was significantly shorter, and treadmill speed, heart rate, RPE and oxygen uptake were all greater in VIG compared with MOD ($P \leq 0.05$). Respiratory exchange ratio was higher in VIG than MOD ($P < 0.001$), with the relative contributions of carbohydrate and fat to energy provision higher and lower, respectively, in VIG compared with MOD (both $P < 0.001$). Gross energy expenditure was not significantly different between the exercise conditions ($P = 0.38$).

Hunger perceptions

Fasting hunger ratings were similar across the conditions at baseline ($P = 0.50$) (Table 3). Linear mixed models revealed no differences in hunger ratings across the conditions (main effect condition $P = 0.35$; main effect time $P < 0.001$; condition by time interaction $P = 0.78$) (Figure 2). Hunger total AUC was similar across the conditions ($P = 0.65$) (Table 3).

Acylated ghrelin, glucose and insulin concentrations

Boxplot analysis of acylated ghrelin total AUC values identified one participant as an outlier (Field 2009). This participant exhibited a mean acylated ghrelin concentration 21 times greater than the mean SD of the remaining participants (range: 74–1489 $pg \cdot mL^{-1}$). Therefore, this participant was removed and results are presented for eight participants. Fasting acylated ghrelin concentrations were similar across the conditions at baseline ($P = 0.57$) (Table 3). Linear mixed models for delta acylated ghrelin revealed a significant main effect of condition ($P < 0.001$), time ($P < 0.001$) and condition by time interaction ($P = 0.03$) (Figure 2). Post-hoc analysis of between-condition differences revealed delta acylated ghrelin concentrations were

lower than control in MOD (ES=0.44, *P*=0.01) and VIG (ES=0.98, *P*<0.001); VIG was lower than MOD (ES=0.54, *P*=0.003). Post-hoc analysis of the condition by time interaction revealed the delta acylated ghrelin concentration was lower than control in VIG at 0.5 h (ES=5.49, *P*=0.005) and 1 h (ES=2.46, *P*=0.02); VIG was lower than MOD at 0.5 h (ES=4.68, *P*=0.02). Delta total AUC for acylated ghrelin was lower in VIG compared with control (ES=2.45, *P*=0.01) (Table 3).

Fasting glucose concentrations were similar across the conditions at baseline (*P*=0.63) (Table 3). Linear mixed models for glucose identified a main effect of condition (*P*=0.02) and time (*P*<0.001), but not a condition by time interaction (*P*=0.46) (Figure 2). Post-hoc analysis of between-condition differences revealed mean VIG glucose concentration was 6% and 5% higher than control (ES=0.31, *P*=0.02) and MOD (ES=0.27, *P*=0.04), respectively; CON and MOD were similar (1%; ES=0.04, *P*=0.73). The VIG glucose AUC was meaningfully, albeit not significantly, higher than control (6%; ES=0.62, *P*=0.09) and MOD (6%; ES=0.58, *P*=0.09); CON and MOD were not different (0%; ES=0.05, *P*=0.86) (Table 3).

Fasting insulin concentrations were similar across the conditions at baseline (*P*=0.19) (Table 3). No differences in insulin concentrations were seen across the conditions (main effect condition *P*=0.28; main effect time *P*<0.001; condition by time interaction *P*=0.26) (Figure 2). Insulin total AUC was similar across the conditions (*P*=0.95) (Table 3).

Correlations

There were no significant correlations between delta acylated ghrelin concentrations and changes in hunger, glucose or insulin values.

Experiment two: exercise duration

Exercise responses

Exercise responses for EX45 and EX90 are displayed in Table 2. The only significant difference was the anticipated increase in gross energy expenditure for EX90 compared with EX45 (P<0.001).

Hunger perceptions

Fasting hunger ratings were similar across the conditions at baseline (P=0.73) (Table 4). Linear mixed models for hunger revealed a significant main effect of condition (P=0.001), time (P<0.001) and condition by time interaction (P<0.001) (Figure 3). Post-hoc analysis of between-condition differences revealed hunger perceptions were 15% and 20% lower than control in EX45 (ES=0.20, P=0.01) and EX90 (ES=0.27, P=0.001), respectively; EX45 and EX90 were similar (-6%; ES=0.07, P=0.34). Post-hoc analysis of the condition by time interaction revealed hunger perceptions were lower than control in EX45 at 0.5, 0.75 and 1 h (all ES≥1.71, P≤0.05); EX90 was lower than control at 0.5, 0.75, 1, 1.5 and 2 h (all ES≥1.30, P≤0.05). The hunger total AUC was 14% and 18% lower than control in EX45 (ES=0.36, P=0.07) and EX90 (ES=0.48, P=0.02), respectively; EX45 and EX90 were similar (-5%; ES=0.13, P=0.42) (Table 4). Specifically, hunger AUC was lower than control between 0–2 h in EX45 (43%; ES=1.96, P=0.001) and EX90 (54%; ES=2.77, P<0.001); EX90 was meaningfully, albeit not significantly, lower than EX45 (20%; ES=0.81, P=0.08).

Acylated ghrelin, glucose and insulin concentrations

Fasting acylated ghrelin concentrations were similar across the conditions at baseline (P=0.88) (Table 4). Linear mixed models for delta acylated ghrelin identified a significant main effect for condition (P<0.001) and time (P<0.001), but not a condition by time interaction (P=0.47)

(Figure 3). Post-hoc analysis of between-condition differences revealed delta acylated ghrelin concentrations were lower than control in EX45 (ES=0.77, $P<0.001$) and EX90 (ES=0.68, $P<0.001$); EX45 and EX90 were similar (ES=0.09, $P=0.55$). The delta total AUC for acylated ghrelin was lower than control in EX45 (ES=0.99, $P=0.03$) and EX90 (ES=0.81, $P=0.07$), respectively; EX45 and EX90 were similar (ES=0.18, $P=0.68$) (Table 4). Specifically, EX45 was lower than control between 0–2 h (ES=1.93, $P<0.001$) and 2–6 h (ES=1.05, $P=0.05$); EX90 was lower than control between 0–2 h (ES=2.16, $P<0.001$) and 2–6 h (ES=0.83, $P=0.18$).

Fasting glucose concentrations were similar across the conditions at baseline ($P=0.98$) (Table 4). Linear mixed models for glucose identified a significant main effect for condition ($P<0.001$), time ($P<0.001$) and condition by time interaction ($P<0.001$) (Figure 3). Post-hoc analysis of between-condition differences revealed mean glucose concentrations were 5% higher than control in EX45 (ES=0.40, $P=0.001$) and EX90 (ES=0.40, $P=0.001$); EX45 and EX90 were similar (0%; ES=0.00, $P=0.97$). Post-hoc analysis of the condition by time interaction revealed the glucose concentration was higher than control in EX45 at 0.75 h (26%; ES=4.17, $P=0.01$). Linear mixed models identified a trend for differences in glucose total AUC across the conditions ($P=0.06$), but post-hoc analysis revealed no significant between-condition differences after Holm-Bonferroni correction ($P\geq0.09$) (Table 4).

Fasting insulin concentrations were similar across the conditions at baseline ($P=0.74$) (Table 4). Linear mixed models for insulin revealed a significant main effect for condition ($P=0.03$) and time ($P<0.001$), but not a condition by time interaction ($P=0.18$) (Figure 3). Post-hoc analysis of between-condition differences revealed mean insulin concentrations were 20% and 25% lower in EX90 than control (ES=0.22, $P=0.08$) and EX45 (ES=0.27, $P=0.03$), respectively; CON and EX45 were similar (6%; ES=0.05, $P=0.61$). Insulin total AUC was not significantly different across the conditions ($P=0.81$) (Table 4).

Correlations

There were no significant correlations between delta acylated ghrelin concentrations and changes in hunger, glucose or insulin values for any time period.

DISCUSSION

The purpose of the present experiments was to elucidate the effect of exercise intensity and duration on acylated ghrelin concentrations and hunger perceptions. The primary findings are that isoenergetic vigorous-intensity running transiently suppressed acylated ghrelin concentrations to a greater extent than moderate-intensity running, but was not accompanied by a change in hunger. Furthermore, acylated ghrelin concentrations and hunger were suppressed to a similar extent during 45 and 90 min treadmill running, but the effect appears prolonged when the exercise duration is extended.

Research has demonstrated that acute exercise suppresses acylated ghrelin concentrations, with perturbations returning to control values within 30 min after exercise (King *et al.* 2013; Schubert *et al.* 2014). Experiment one extends these findings by demonstrating that acylated ghrelin concentrations were reduced to a greater extent during vigorous-intensity running than moderate-intensity running, despite a similar exercise-induced energy expenditure. This is consistent with previous research identifying exercise intensity as an important determinant of the acylated ghrelin response to acute exercise, with suppression occurring at intensities $\geq$60% $\dot{V}O_{2peak}$ typically (Broom *et al.* 2007, 2009; Ueda *et al.* 2009; King *et al.* 2010a, 2010b). The importance of exercise intensity is highlighted further by studies reporting that sprint interval exercise suppresses acylated ghrelin to a greater extent than moderate-intensity exercise (Deighton *et al.* 2013; Metcalfe *et al.* 2015). However, studies directly comparing isoenergetic bouts of moderate- and vigorous-to-high-intensity exercise have reported contrasting findings, with one study reporting greater suppression of acylated ghrelin at the

higher exercise intensity (akin to experiment one) (Sim *et al.* 2014), whilst others demonstrate a similar level of suppression independent of exercise intensity (Martins *et al.* 2015; Howe *et al.* 2016). The discrepancy in findings is likely related to key variations in the protocols adopted including differences in the participant groups, exercise energy expenditure, completion of exercise in the fasted or postprandial state and timing of meal intake. Differences in meal size and macronutrient composition, and methods utilised to quantify acylated ghrelin are likely to further confound the interpretation of these findings. Additional work is clearly required to elucidate the impact of exercise intensity on acylated ghrelin.

Surprisingly, despite the decrease in acylated ghrelin during vigorous-intensity exercise, hunger did not differ significantly between conditions. Although this contrasts previous studies reporting simultaneous reductions in acylated ghrelin and hunger in response to exercise (Broom *et al.* 2007, 2009; King *et al.* 2010a), exercise-induced changes in acylated ghrelin and hunger do not always occur in parallel (Deighton *et al.* 2013; Sim *et al.* 2014; Martins *et al.* 2015). This apparent disassociation highlights the complex nature of appetite regulation, which involves the interaction of many physiological and psychological factors (Hussain & Bloom 2013).

In accordance with previous studies, experiment two demonstrated a reduction in acylated ghrelin concentrations and hunger in both exercise conditions (Broom *et al.* 2007, 2009; King *et al.* 2010a). Although the hunger and acylated ghrelin responses were not statistically different between the two exercise interventions, the values remained suppressed at 1.5 h in the 90 min, but not 45 min, exercise bout (Figure 3). This suggests that increasing the exercise duration may extend the exercise-induced suppression in hunger and acylated ghrelin concentrations. Although this is the first study to investigate the effect of exercise duration on acylated ghrelin concentrations, Erdmann *et al.* (2007) reported no differences in total ghrelin concentrations in response to 30, 60 and 120 min cycling. However, acylated

ghrelin is the form of ghrelin thought to be solely responsible for appetite stimulation (Ghigo *et al.* 2005), and may be obscured when total ghrelin is measured (Hosoda *et al.* 2004). Furthermore, research examining the effect of acute exercise on total ghrelin concentrations has yielded equivocal findings with evidence of acute increases, decreases and no change (King *et al.* 2013).

Similar to experiment one, there was a divergence in the acylated ghrelin and hunger responses to acute exercise, further highlighting the complexity of appetite regulation. Although simultaneous reductions in acylated ghrelin and hunger were seen during exercise, hunger ratings returned to similar values between conditions at 2.5 h, but acylated ghrelin remained suppressed in the exercise conditions after meal consumption. The reason for this disparity is unclear but the findings of the present experiments contribute to the debate concerning the importance of reductions in acylated ghrelin as a potential determinant of hunger.

The physiological significance of transient reductions in acylated ghrelin during and after exercise is not fully understood. The divergence between acylated ghrelin and hunger demonstrated in the present experiments and previous studies (Deighton *et al.* 2013; Sim *et al.* 2014; Martins *et al.* 2015) challenges the role acylated ghrelin plays in mediating appetite responses to exercise. Furthermore, although the implementation of standardised meals in the present experiments precluded the assessment of energy intake, the consensus of evidence suggests that acute aerobic exercise does not stimulate compensatory increases in appetite and energy intake on the same day (Deighton & Stensel 2014). This may point to the existence of alternative compensatory mechanisms, for example, reductions in unstructured physical activity (i.e., non-exercise activity thermogenesis) and/or increased sedentary behaviours on the day of exercise, but further work is required to support this. Nevertheless, acylated ghrelin is the only gut peptide known to stimulate appetite and energy intake, with

circulating concentrations increasing preprandially and decreasing postprandially on a meal-to-meal basis (Cummings *et al.* 2004). Consequently, this temporal pattern of fluctuation in acylated ghrelin is indicative of an important role in coordinating meal initiation and/or termination (Cummings *et al.* 2004; Kara & Batterham 2010).

The mechanisms underpinning the transient exercise-induced suppression of acylated ghrelin are unclear but are likely to reflect processes interfering with the synthesis and/or secretion of acylated ghrelin into the circulation. A recent review suggests the redistribution of blood flow from splanchnic areas to active skeletal muscle may be particularly pertinent for suppressing ghrelin, and appear dependent on the exercise intensity (Hazell *et al.* 2016). Exercise-induced changes in glucose and insulin concentrations have also been implicated mechanistically (Hazell *et al.* 2016), with elevations associated with decreased ghrelin concentrations (Flanagan *et al.* 2003; Cummings & Overduin 2007; Iwakura *et al.* 2015). The elevation in glucose concentration during vigorous-intensity exercise in experiment one and both exercise conditions in experiment two coincided with the reduction in acylated ghrelin concentrations. However, insulin concentrations were reduced in the 90 min exercise condition, and previous exercise studies provide conflicting findings by reporting no effect of glucose and insulin on acylated ghrelin concentrations (Broom *et al.* 2007, 2009; King *et al.* 2010a). Further research is required to develop a mechanistic understanding of the exercise-induced suppression of acylated ghrelin.

One limitation of the present experiments represents the measurement of a single appetite-regulating hormone. Despite the unique role of acylated ghrelin as the only appetite-stimulating gut hormone, it is only one component of the appetite-regulating neuroendocrine system. Therefore, it may be prudent for future studies to investigate anorexigenic hormones (e.g., peptide-YY, glucagon-like peptide-1, pancreatic polypeptide and cholecystokinin) to provide a broader scientific understanding of the role exercise intensity and duration play in

modulating appetite regulation. Secondly, appetite perceptions were limited to the assessment of hunger; however, utilising multiple scales (e.g., satisfaction, fullness and prospective food consumption) may provide a more holistic insight into appetite perceptions (Blundell *et al.* 2010). Finally, we recruited a small group of healthy and recreationally active men to both experiments, which may limit applications to other population groups and the ability to detect meaningful associations between variables. Additional research is needed in overweight and obese populations who are most likely to benefit from weight management strategies. Despite these limitations, our findings provide important insight into the role that exercise intensity and duration play in modulating hormonal and hunger responses to exercise.

In conclusion, the present experiments demonstrate that exercise intensity, and to a lesser extent duration, are determinants of the acylated ghrelin response to exercise. Acylated ghrelin is transiently suppressed after a bout of exercise, an effect that appears greater when exercise is performed at a higher intensity. Increasing the exercise duration may prolong the transient suppression in hunger and acylated ghrelin, but the disassociation between hunger and acylated ghrelin responses requires further investigation. Future research is warranted to examine these responses chronically and in overweight/obese populations for whom exercise may be a therapeutic strategy for weight management.

Declaration of interest

The authors declare no conflict of interest.

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448 **References**

449 Atkinson G 2002 Analysis of repeated measurements in physical therapy research: multiple
450 comparisons amongst level means and multi-factorial designs. *Physical Therapy in Sport* **3**
451 191–203.

452 Blundell J, de Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, Mela D, Salah S,
453 Schuring E, van der Knaap H *et al*. 2010 Appetite control: methodological aspects of the
454 evaluation of foods. *Obesity Reviews* **11** 251–270.

455 Borg GA 1973 Perceived exertion: a note on ‘‘history’’ and methods. *Medicine and Science*
456 *in Sports* **5** 90–93.

457 Broom DR, Stensel DJ, Bishop NC, Burns SF & Miyashita M 2007 Exercise-induced
458 suppression of acylated ghrelin in humans**.** *Journal of Applied Physiology* **102** 2165–2171.

459 Broom DR, Batterham RL, King JA & Stensel DJ 2009 Influence of resistance and aerobic
460 exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males.
461 *American Journal of Physiology. Regulatory Integrative and Comparative Physiology* **296**
462 R29–R35.

463 Cohen J 1988 *Statistical power analysis for the behavioural sciences*, edn 2, pp 22–25.
464 Hillsdale (NJ): Lawrence Erlbaum Associates.

465 Cummings DE, Frayo RS, Marmonier C, Aubert R & Chapelot D 2004 Plasma ghrelin levels
466 and hunger scores in humans initiating meals voluntarily without time- and food-related cues.
467 *American Journal of Physiology. Endocrinology and Metabolism* **287** E297–E304.

468 Cummings DE & Overduin J 2007 Gastrointestinal regulation of food intake. *The Journal of*
469 *Clinical Investigation* **117** 13–23.

Deighton K, Barry R, Connon CE & Stensel DJ 2013 Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *European Journal of Applied Physiology* **113** 1147–1156.

Deighton K & Stensel DJ 2014 Creating an acute energy deficit without stimulating compensatory increases in appetite: is there an optimal exercise protocol? *Proceedings of the Nutrition Society* **73** 352–358.

Dill DB & Costill DL 1974 Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology* **37** 247–248.

Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW & Smith BK 2009 American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and Science in Sports and Exercise* **41** 459–471.

Erdmann J, Tahbaz R, Lippl F, Wagenpfeil S & Schusdziarra V 2007 Plasma ghrelin levels during exercise – effects of intensity and duration. *Regulatory Peptides* **143** 127–135.

Field A 2009 *Discovering statistics using SPSS*, edn 3. London: Sage.

Flanagan DE, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV & Sherwin RS 2003 The influence of insulin on circulating ghrelin. *American Journal of Physiology. Endocrinology and Metabolism* **284** E313–E316.

Flint A, Raben A, Blundell JE & Astrup A 2000 Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* **24** 38–48.

492 Frayn KN 1983 Calculation of substrate oxidation rates in vivo from gaseous exchange.
493 *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* **55** 628–
494 634.

495 Ghigo E, Broglio F, Arvat E, Maccario M, Papotti M & Muccioli G 2005 Ghrelin: more than
496 a natural GH secretagogue and/or an orexigenic factor. *Clinical Endocrinology* **62** 1–17.

497 Hazell TJ, Islam H, Townsend LK, Schmale MS & Copeland JL 2016 Effects of exercise
498 intensity on plasma concentrations of appetite-regulating hormones: potential mechanisms.
499 *Appetite* **98** 80–88.

500 Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E, Enomoto M, Ono F & Kangawa K
501 2004 Optimum collection and storage conditions for ghrelin measurements: octanoyl
502 modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clinical*
503 *Chemistry* **50** 1077–1080.

504 Howe SM, Hand TM, Larson-Meyer DE, Austin KJ, Alexander BM & Manore MM 2016 No
505 effect of exercise intensity on appetite in highly-trained endurance women. *Nutrients* **8** E223.

506 Hussain SS & Bloom SR 2013 The regulation of food intake by the gut-brain axis:
507 implications for obesity. *International Journal of Obesity* **37** 625–633.

508 Iwakura H, Kangawa K & Nakao K 2015 The regulation of circulating ghrelin – with recent
509 updates from cell-based assays. *Endocrine Journal* **62** 107–122.

510 Karra E & Batterham RL 2010 The role of gut hormones in the regulation of body weight and
511 energy homeostasis. *Molecular and Cellular Endocrinology* **316** 120–128.

King NA, Burley VJ & Blundell JE 1994 Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition* **48** 715–724.

King JA, Miyashita M, Wasse LK & Stensel DJ 2010a Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* **54** 492–498.

King JA, Wasse LK, Broom DR & Stensel DJ 2010b Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Medicine and Science in Sports and Exercise* **42** 485–492.

King JA, Wasse LK, Stensel DJ & Nimmo MA 2013 Exercise and ghrelin. A narrative overview of research. *Appetite* **68** 83–91.

Martins C, Stensvold D, Finlayson G, Holst J, Wisloff U, Kulseng B, Morgan L & King NA 2015 Effect of moderate- and high-intensity acute exercise on appetite in obese individuals. *Medicine and Science in Sports and Exercise* **47** 40–48.

Metcalfe RS, Koumanov F, Ruffino JS, Stokes KA, Holman GD, Thompson D & Vollaard NBJ 2015 Physiological and molecular responses to an acute bout of reduced-exertion high-intensity interval training (REHIT). *European Journal of Applied Physiology* **115** 2321–2334.

Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF *et al*. 2014 Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384** 766–781.

Schubert MM, Sabapathy S, Leveritt M & Desbrow B 2014 Acute exercise and hormones related to appetite regulation: a meta-analysis. *Sports Medicine* **44** 387–403.

535 Sim AY, Wallman KE, Fairchild TJ & Guelfi KJ 2014 High-intensity intermittent exercise
536 attenuates *ad-libitum* energy intake. *International Journal of Obesity* **38** 417–422.

537 Taylor HL, Buskirk E & Henschel A 1955 Maximal oxygen intake as an objective measure of
538 cardio-respiratory performance. *Journal of Applied Physiology* **8** 73–80.

539 Ueda SY, Yoshikawa T, Katsura Y, Usui T, Nakao H & Fujimoto S 2009 Changes in gut
540 hormone levels and negative energy balance during aerobic exercise in obese young males.
541 *Journal of Endocrinology* **201** 151–159.

542 Wang YC, McPherson K, Marsh T, Gortmaker SL & Brown M 2011 Health and economic
543 burden of the projected obesity trends in the USA and the UK. *Lancet* **378** 815–825.

Figure legends

Figure 1 Schematic representation of the study protocol in experiment one and two.

Figure 2 Perceptions of hunger (A), and concentrations of plasma delta acylated ghrelin (B), insulin (C) and glucose (D) in the control (■), moderate-intensity exercise (●) and vigorous-intensity exercise (△) conditions. Values are mean (SEM), n = 9 for hunger, insulin and glucose and n = 8 for acylated ghrelin. Black rectangle indicates moderate-intensity exercise, grey rectangle indicates vigorous-intensity exercise and open rectangle indicates consumption of the standardised meal.

Figure 3 Perceptions of hunger (A), and concentrations of plasma delta acylated ghrelin (B), insulin (C) and glucose (D) in the control (■), 45 min exercise (●) and 90 min exercise (△) conditions. Values are mean (SEM), n = 9 for hunger, acylated ghrelin, insulin and glucose. Black rectangle indicates 90 min exercise, grey rectangle indicates 45 min exercise and open rectangles indicates consumption of the standardised meals.

Table 1 Physical and physiological characteristics in experiments one and two.

Characteristic	**Experiment one (*n* = 9)**	**Experiment two (*n* = 9)**
Age (years)	21.4 (1.7)	23.2 (2.1)
Body mass (kg)	78.3 (11.0)	72.0 (5.6)
Stature (m)	1.79 (0.07)	1.78 (0.05)
Body mass index ($kg \cdot m^{-2}$)	24.5 (2.4)	22.7 (1.5)
Sum of skinfolds (mm)	33.1 (5.7)	26.1 (4.5)
Percent body fat (%)	15.3 (2.7)	12.0 (2.3)
Waist circumference (cm)	77.7 (5.7)	76.7 (2.1)
Peak oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$)	58 (6)	63 (6)

Values are mean (SD)

Table 2 Responses to treadmill running in experiments one and two.

	Experiment one: exercise intensity			Experiment two: exercise duration		
	Moderate-intensity	**Vigorous-intensity**	**Effect size**	**45 min**	**90 min**	**Effect size**
Exercise time (min)	55 (7)	36 (5)	2.97[a]	45 (0)	90 (0)	-
Treadmill speed ($km \cdot h^{-1}$)	7.5 (0.8)	11.0 (1.5)	2.84[a]	10.6 (1.4)	10.4 (1.3)	0.09
Heart rate ($beats \cdot min^{-1}$)	136 (15)	163 (19)	1.57[a]	169 (11)	169 (12)	0.00
Rating of perceived exertion	12 (1)	14 (2)	1.49[a]	13 (1)	14 (1)	0.59
Oxygen uptake ($L \cdot min^{-1}$)	2.37 (0.35)	3.41 (0.40)	2.74[a]	3.19 (0.36)	3.17 (0.34)	0.06
Percent peak oxygen uptake (%)	52 (3)	75 (4)	6.32[a]	70 (2)	70 (2)	0.20
Respiratory exchange ratio	0.90 (0.03)	0.96 (0.04)	1.95[a]	0.93 (0.05)	0.89 (0.11)	0.52
Fat oxidation (%)	32 (9)	11 (10)	2.26[a]	24 (10)	33 (27)	0.44
Carbohydrate oxidation (%)	68 (9)	89 (10)	2.26[a]	76 (10)	67 (27)	0.44
Gross energy expenditure (kJ)	2580 (152)	2504 (165)	0.48	2918 (329)	5949 (653)	5.86[b]

Values are mean (SD)

[a] Significant difference between moderate-intensity exercise and vigorous-intensity exercise ($P < 0.05$)

[b] Significant difference between 45 min exercise and 90 min exercise ($P < 0.05$)

Table 3 Fasting and time-averaged total area under the concentration versus time curve in the control, moderate-intensity exercise and vigorous-intensity exercise conditions in experiment one.

	Control	Moderate-intensity exercise	Vigorous-intensity exercise	Main effect condition *P*
Hunger				
Fasting (mm)	33 (18 to 60)	30 (16 to 55)	25 (14 to 46)	0.50
TAUC (mm)	53 (44 to 65)	49 (41 to 60)	51 (42 to 61)	0.65
Acylated ghrelin				
Fasting ($pg \cdot mL^{-1}$)	67.2 (31.4)	68.1 (25.9)	78.9 (42.0)	0.57
Delta TAUC ($pg \cdot mL^{-1}$)	2.29 (8.21)	-6.83 (11.76)	-17.78 (19.16)	0.01[a]
Glucose				
Fasting ($mmol \cdot L^{-1}$)	5.21 (4.68 to 5.80)	5.44 (4.89 to 6.07)	5.52 (4.96 to 6.15)	0.63
TAUC ($mmol \cdot L^{-1}$)	5.20 (4.87 to 5.54)	5.22 (4.89 to 5.57)	5.52 (5.17 to 5.89)	0.06
Insulin				
Fasting ($pmol \cdot L^{-1}$)	137 (107 to 175)	175 (137 to 224)	168 (131 to 215)	0.19
TAUC ($pmol \cdot L^{-1}$)	297 (238 to 371)	292 (234 to 365)	302 (242 to 377)	0.95

Values for acylated ghrelin are mean (SD) for $n = 8$. Values for hunger, glucose and insulin are geometric mean (95% confidence interval) for $n = 9$, and statistical analyses are based on natural log transformed data. TAUC, time-averaged total area under the concentration versus time curve.

[a] Significant difference between vigorous-intensity exercise and control conditions (linear mixed model $P < 0.05$ after Holm-Bonferroni correction)

Table 4 Fasting and time-averaged total area under the concentration versus time curve in the control, 45 min exercise and 90 min exercise conditions in experiment two.

	Control	45 min exercise	90 min exercise	Main effect condition *P*
Hunger				
Fasting (mm)	45 (30 to 68)	47 (31 to 72)	43 (28 to 65)	0.73
TAUC (mm)	37 (27 to 49)	31 (23 to 42)	30 (22 to 40)	0.02[a]
Acylated ghrelin				
Fasting ($pg \cdot mL^{-1}$)	159 (140)	163 (140)	153 (128)	0.88
Delta TAUC ($pg \cdot mL^{-1}$)	-7.44 (48.30)	-55.20 (77.34)	-46.56 (53.75)	0.07
Glucose				
Fasting ($mmol \cdot L^{-1}$)	5.04 (4.78 to 5.32)	5.06 (4.80 to 5.34)	5.04 (4.78 to 5.32)	0.98
TAUC ($mmol \cdot L^{-1}$)	5.05 (4.88 to 5.23)	5.32 (5.14 to 5.50)	5.35 (5.17 to 5.54)	0.06
Insulin				
Fasting ($pmol \cdot L^{-1}$)	21.3 (12.1 to 37.7)	19.6 (11.1 to 34.6)	17.1 (9.7 to 30.2)	0.74
TAUC ($pmol \cdot L^{-1}$)	67.8 (47.7 to 96.5)	68.2 (47.9 to 97.1)	61.5 (43.2 to 87.5)	0.81

Values for acylated ghrelin are mean (SD) for $n = 9$. Values for hunger, glucose and insulin are geometric mean (95% confidence interval) for $n = 9$, and statistical analyses are based on natural log transformed data. TAUC, time-averaged total area under the concentration versus time curve.

[a] Significant difference between 90 min exercise and control conditions (linear mixed model $P < 0.05$ after Holm-Bonferroni correction)

Figure 1

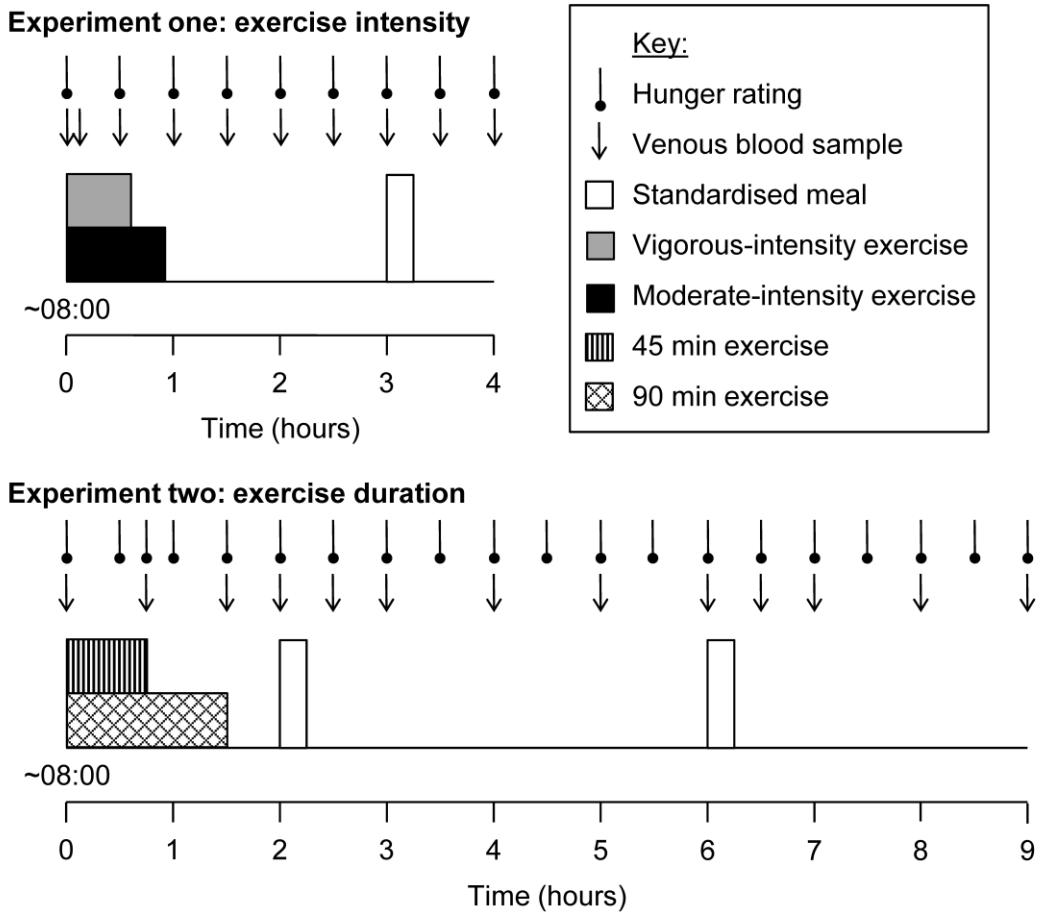

Experiment one: exercise intensity
Key:
Hunger rating
Venous blood sample
Standardised meal
Vigorous-intensity exercise
Moderate-intensity exercise
45 min exercise
90 min exercise
~08:00
0
1
2
3
4
Time (hours)
Experiment two: exercise duration
~08:00
0
1
2
3
4
5
6
7
8
9
Time (hours)

Figure 2

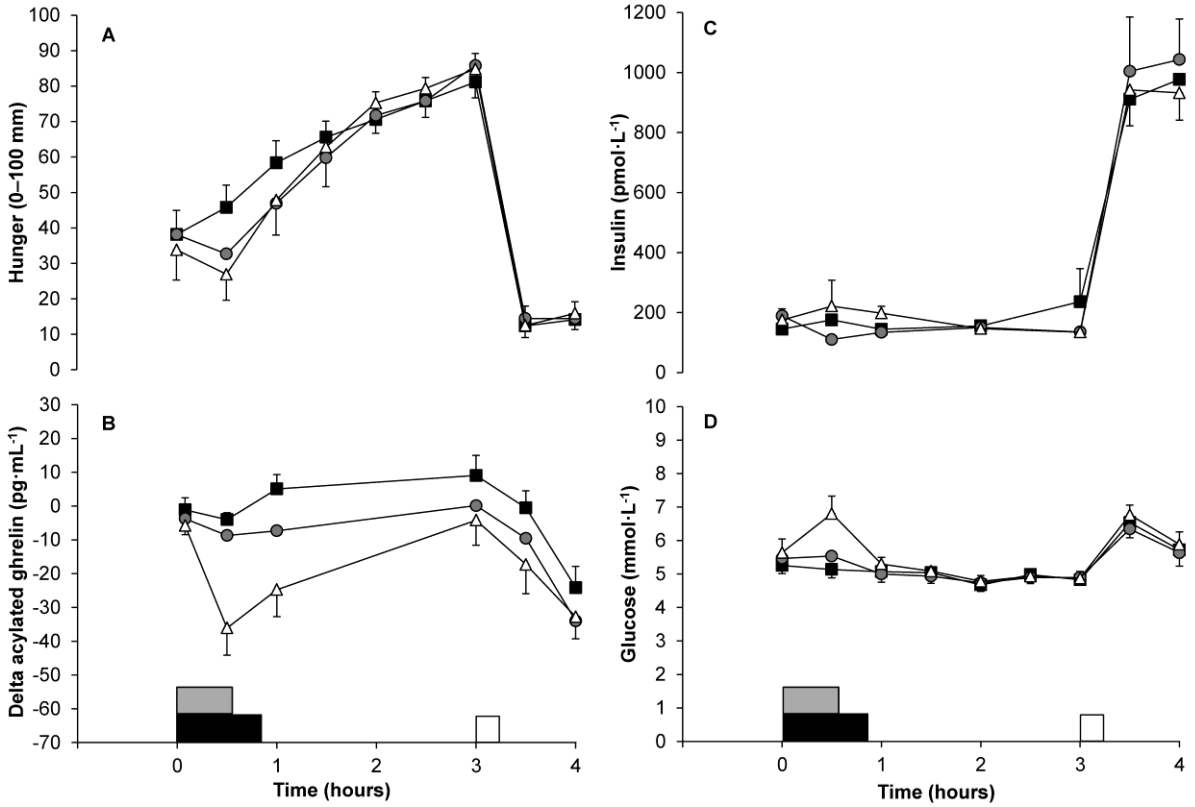
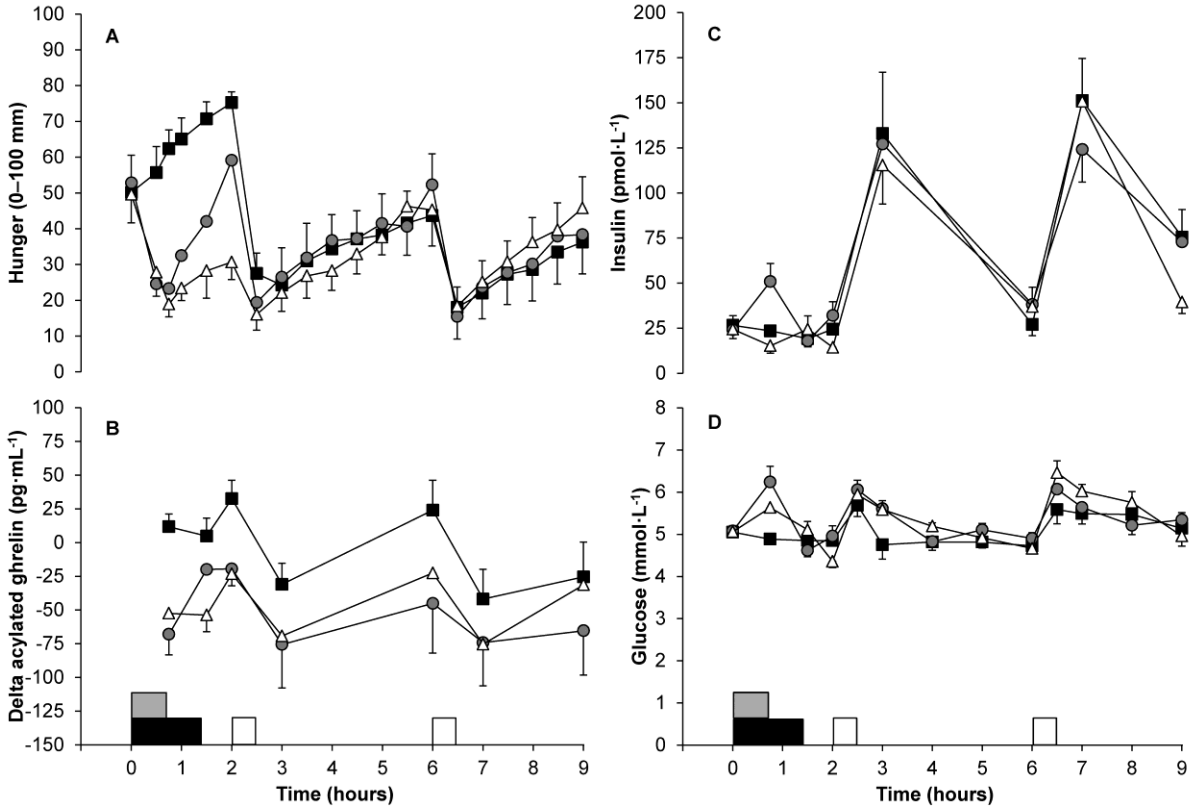

A
Hunger (0–100 mm)
B
Delta acylated ghrelin (pg·mL-1)
C
Insulin (pmol·L-1)
D
Glucose (mmol·L-1)
Time (hours)
Time (hours)

Figure 3


A
Hunger (0–100 mm)
B
Delta acylated ghrelin (pg·mL-1)
C
Insulin (pmol·L-1)
D
Glucose (mmol·L-1)
Time (hours)
Time (hours)