Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration

Dr Adrian Holliday[1,2], and Dr. Andrew Blannin[1]

[1]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham. United Kingdom. B15 2TT.

[2]Carnegie School of Sport, Leeds Beckett University, Headingley Campus, Leeds. United Kingdom. LS6 3QS.

Corresponding Author:

Dr Adrian Holliday

Fairfax G18,

Headingley Campus,

Leeds Beckett University,

Leeds.

LS6 3QS

a.j.holliday@leedsbeckett.ac.uk

Short title: *Exercise duration effects on appetite measures*

Keywords: Appetite hormones; eating behaviour; post-exercise nutrition

Word count: 5727

Footnote. Dr Adrian Holliday's current affiliation: School of Sport, Leeds Beckett University, Headingley Campus, Leeds. LS6 3QS.

ABSTRACT (250 words)

Purpose: To investigate the effect of acute bouts of high-intensity aerobic exercise of differing durations on subjective appetite, food intake and appetite-associated hormones in endurance-trained males.

Method: Twelve endurance-trained males (age=21±2 years; BMI=21.0±1.6 kg•m^{-2}; VO_{2max}=61.6±6.0 mL• kg^{-1}• min^{-1}) completed four trials, within a maximum 28-day period, in a counterbalanced order: resting (REST); 15-minutes exercise bout (15-MIN); 30-minute exercise bout (30-MIN) and 45-minute exercise bout (45-MIN). All exercise was completed on a cycle ergometer at an intensity of ~76% VO_{2max}. Sixty minutes post-exercise, participants consumed an *ad libitum* meal. Measures of subjective appetite and blood samples were obtained throughout the morning, with plasma analysed for acylated ghrelin, total polypeptide tyrosine-tyrosine (PYY) and total glucagon-like peptide 1 (GLP-1) concentrations.

Results: Neither subjective appetite nor absolute food intake differed between trials. Relative energy intake (intake – expenditure) was significantly greater after REST (2641±1616 kJ) compared with both 30-MIN (1039±1520 kJ) and 45-MIN (260±1731 kJ), and significantly greater after 15-MIN (2699±1239 kJ) compared with 45-MIN (condition main effect, $p<0.001$). GLP-1 concentration increased immediately post-exercise in 30-MIN and 45-MIN, respectively (condition-x-time interaction, $p<0.001$). Acylated ghrelin was transiently suppressed in all exercise trials (condition-x-time interaction, $p=0.011$); the greatest, most enduring suppression was observed in 45-MIN. PYY concentration was unchanged with exercise.

Conclusion: High-intensity aerobic cycling lasting up to 45 minutes did not suppress subjective appetite or affect absolute food intake, but did reduce relative energy intake, in well-trained endurance athletes. Findings question the role of appetite hormones in regulating subjective appetite in the acute post-exercise period.

ABBREVIATIONS

15-MIN, 15-minute exercise condition

30-MIN, 30-minute exercise condition

45-MIN, 45-minute exercise condition

DEBQ, Dutch Eating Behaviour Questionnaire

GLP-1, glucagon-like peptide 1

PYY, polypeptide tyrosine-tyrosine

REI, relative energy intake

REST, resting condition

VAS, Visual analogue scale

WORD COUNT: 5727

INTRODUCTION

High-intensity aerobic exercise (≥60% VO_{2max}) commonly elicits a transient suppression of appetite in lean, recreationally active individuals (Broom *et al.*, 2007, Martins *et al.*, 2007, Broom *et al.*, 2009, Ueda *et al.*, 2009b, Ueda *et al.*, 2009a, King *et al.*, 2010). This phenomenon, termed the "anorexia of exercise" (King *et al.*, 1994), is often coupled with anorexigenic changes in appetite-associated hormones (Schubert *et al.*, 2014).

While the exercise intensity dependency of post-exercise appetite suppression appears well established, the effect of the duration of exercise is yet to be comprehensively investigated. Suppressions in appetite, accompanied by increases in the plasma concentration of satiety peptides peptide tyrosine-tyrosine (PYY) and glucagon-like peptide 1 (GLP-1) have been observed with continuous, high-intensity aerobic bouts of exercise lasting as little as 30 minutes (Ueda et al., 2009a), and with intermittent exercise bouts yielding energy expenditure values of as little as ~150 kcal (~628 kJ) (Deighton *et al.*, 2013). Conversely, bouts of very low energy cost (~51 kcal (213kJ)) have elicited increases in subjective appetite (Bellissimo *et al.*, 2007). In contrast, appetite has been shown to be unaffected after continuous exercise bouts lasting as long as 90 minutes (King *et al.*, 2011a). When directly comparing exercise of different duration, Erdman et al (Erdmann *et al.*, 2007) observed an increase in total ghrelin with low intensity exercise (cycling at 50W), lasting 30, 60 and 120 minutes, that was not duration dependent. Similarly, Broom and colleagues (Broom *et al.*, 2017) observed a comparable immediate post-exercise suppression of hunger and acylated ghrelin after 45 minutes and 90 minutes of aerobic exercise at 70% VO_{2max}; however, the suppression was more enduring after the 90-minute bout. It remains unknown whether any of the appetite-associated hormones are released in a dose-response manner to exercise duration or energy cost, or whether there is a duration or energy cost threshold for a hormonal response.

The transient nature of both a suppression of subjective appetite and changes in plasma appetite-associated hormone concentration means that *ad libitum* food intake can be reduced when administered in close proximity to the cessation of exercise (~10 minutes (Westerterp-Plantenga *et al.*, 1997); ~15 minutes (Kissileff *et al.*, 1990); ~30 minutes (Ueda *et al.*, 2009a); ~60 minutes (Ueda *et al.*, 2009b)), but is largely unaffected when a meal is consumed ≥60 minutes after exercise (Thompson *et al.*, 1988, King *et al.*, 1997, Martins *et al.*, 2007, King *et al.*, 2010, King *et al.*, 2011b, Schubert *et al.*, 2013).

The majority of previous studies have used study populations of recreationally active individuals, and the study of trained individuals is limited (Howe *et al.*, 2016). Trained endurance athletes regularly complete very strenuous bouts of exercise that are of high intensity, long duration and continuous in nature. It is yet to be

confirmed whether appetite responses to such strenuous bouts in athletic populations is akin to exercise of a less strenuous nature in untrained individuals. It is possible that a more strenuous bout of exercise may elicit a greater and more enduring appetite suppression.

Any post-exercise appetite suppression could have implications for trained athletes. Post-exercise nutrition is often considered of crucial importance to optimise recovery and maximise adaptations to training (Burke, 1997). In addition, many athletes value weight management highly (Filaire *et al*., 2007, Sundgot-Borgen *et al*., 2013), as an increase in body mass can result in an increase in the energy cost of performing. Nevertheless, few investigations have addressed the effect of exercise on any appetite-related measures in athletic populations. Both increases (Jurimae *et al*., 2003, Jurimae *et al*., 2005, Jurimae *et al*., 2006, Jürimäe *et al*., 2007, Jurimae *et al*., 2009, O'Connor *et al*., 1995, O'Connor *et al*., 2006) and decreases (Jurimae *et al*., 2003, Jürimäe *et al*., 2005) in anorexigenic gut hormones with strenuous exercise have been observed, while increases in the orexigenic hormone ghrelin have also been reported (Jurimae *et al.*, 2007, Jurimae *et al*., 2009). These data suggest that changes in the concentration of appetite-associated hormones in response to high-intensity aerobic exercise may be affected by training status. However, it has yet to be investigated whether this translates to altered appetite and food intake responses.

The purpose of the current study was to address the effect of exercise duration on subjective appetite, food intake and circulating concentrations of acylated ghrelin, total PYY and total GLP-1 in trained endurance athletes, utilising high-intensity exercise bouts, akin to the habitual training of endurance athlete, lasting 15, 30 and 45 minutes.

It was hypothesised that exercise would elicit a transient suppression of appetite in a dose-response fashion, with longer duration of exercise resulting in more enduring appetite suppression. It was surmised that this would be accompanied by anorexigenic changes to appetite-associated hormones. An enduring appetite suppression with greater exercise load of the 45 minute condition may lead to a lower post-exercise energy intake.

MATERIALS AND METHODS

Participants

Twelve endurance trained male athletes were recruited for the study (see **Table 1)**. Inclusion criteria were: a minimum total training duration of 6 hours per week, habitual breakfast eaters, self-reported weight stable for the past 6 months, and aged between 18 and 40 years. Exclusion criteria were: a score of 3.5 or greater

for restricted eating on the Dutch Eating Behaviour Questionnaire (DEBQ, (van Strien *et al.*, 1986)); illness such as upper respiratory tract infections; smoking and the taking of medication likely to affect appetite or induce weight-loss. Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham.

(Table 1)

Study design

Using a within-subject, counterbalanced, crossover study design, participants were randomly assigned to each trial condition: resting (REST), 15 minutes of cycling exercise (15-MIN), 30 minutes of cycling exercise (30-MIN), and 45 minutes of cycling exercise (45-MIN). Exercise was completed at an intensity of ~80% VO_{2max}, with measures of subjective appetite and circulating hormone concentrations recorded throughout each trial.

Pre-testing

A single session of pre-testing preceded study trials in order to calculate the specific intensity of exercise for each participant. Participants reported to the Exercise Metabolism Laboratory, in the School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham after a minimum 2 hour fast and having refrained from strenuous exercise during the previous 48 hours. The participant information pack was administered and explained, and the participant was given the opportunity to ask any questions regarding the study. Informed written consent was obtained before the completion of a health questionnaire and the DEBQ, which was used to assess the participants' habitual degree of eating restraint. Height and weight were recorded. An incremental exhaustive exercise test was completed to obtain VO_{2max}. The exercise test was carried out on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The test, preceded by a ten minute warm-up at a self-selected power output, consisted of 3 minute stages, starting at a power output of 95W and increasing in increments of 35W. Breath-by-breath measures of exhaled gas, averaged every eight breaths, were recorded using Oxycon Pro (Jaeger, Wuerzburg, Germany) apparatus. Participants were adjudged to have reached the end of the test when they voluntarily stopped pedalling, if their cadence dropped to <60 rpm or if VO_2 or heart rate ceased to increase with increasing workload. VO_{2max} was calculated as the highest mean value obtained for any 1 minute period. Submaximal VO_2 values were obtained for each stage by disregarding data from the first 2 minutes of the stage. From the VO_{2max} value obtained, linear regression was used to

calculate the work output (in Watts) which would equate to an exercise intensity of 80% VO_{2max}. This value was used for each of the three exercise trials.

Procedure & protocol

A minimum period of 3 days separated the pre-testing session and the first of four study trials. Participants were asked to refrain from moderate or high intensity exercise during the 24h prior to each trial. A food diary was completed for the 24h prior to the first trial, with participants asked to replicate food intake as closely as possible for the 24h prior to subsequent trials. There was a minimum wash out period of 3 days between trials, but typically trials were separated by 7 days.

Participants arrived at the laboratory at approximately 08:00, after a minimum 10-hour overnight fast. On arrival, and after voiding, participants were weighed (body mass was recorded at each visit to ensure participants were weight-stable throughout). A resting blood sample was obtained following the insertion of a venous cannula into the antecubital vein, prior to the measure of baseline subjective appetite.

The exercise bout then commenced. Exercise consisted of cycling on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) at a target intensity of 80% VO_{2max}, for a duration of 15, 30 or 45 minutes. During each exercise bout, exhaled gas samples were obtained intermittently to monitor VO_2 and to retrospectively calculate energy expenditure. Initial monitoring during minutes 3 and 4 allowed the ergometer resistance to be adjusted to achieve the target VO_2. A blood sample was obtained at the half way point of the exercise trial. At 5 minute intervals throughout the exercise bout, measures of heart rate and perceived exertion were obtained. The mean of these values were calculated for the entire bout.

Exercise ceased upon reaching the exercise duration target. A blood sample was obtained and measures of subjective appetite were completed immediately post-exercise. The 60 min rest period then commenced, during which the participant sat reading or watching television. Subjective appetite measures and blood samples were collected at 20, 40 and 60 minutes post-exercise. In REST, the participant remained sedentary throughout, resting for an additional 30 minutes to equate to the median duration of time spent exercising in the three exercise trials.

At 60 minutes post-exercise, the participant consumed an *ad libitum* breakfast meal. This consisted of a buffet, offering the following food: cornflakes, semi-skimmed milk, sugar, bread, margarine, strawberry jam, orange juice and apple juice (nutritional information shown in Appendix 1). All food was pre-weighed and presented in excess. After volitional satiation was reached, the remaining food was weighed and subtracted

from the known quantity provided, allowing for determination of consumed food. From this, energy intake was calculated. As the macronutrient content of each food item was known, absolute (grams) and relative (percentage of total energy) macronutrient intake was also calculated.

Measures

Post-exercise energy intake was assessed using the *ad libitum* breakfast test meal, as described above. A carbohydrate-rich breakfast meal was selected and participants were screened prior to enrolment in the study to ensure that they habitually consumed breakfasts of a similar composition. Participants were allocated a maximum of 15 minutes in which to complete the meal. Subjective appetite was assessed using the 4-question, 150mm, visual analogue scale (VAS) test for subjective appetite, as adapted from Hill & Blundell (Hill & Blundell, 1982). Measures of "hunger," "fullness," "desire to eat," and "expected food intake" were obtained. A composite appetite score was calculated to simplify data analysis and presentation (Holliday & Blannin, 2017). (Composite appetite score = hunger score + desire score + expected intake score + (150-fullness score). The fullness score was reversed due to its opposing direction to the other three questions).

Blood sampling and analysis

All blood samples were immediately transferred to disodium EDTA-treated tubes for analysis of appetite-associated hormones. For the measure of PYY, GLP-1 and acylated ghrelin concentrations, test tubes were pre-treated with the protease inhibitors DPP IV inhibitor (Millipore, MA, USA) and 4- (2 – Aminoethyl) benzenesulfonylfluoride hydrochloride (AEBSF, Alexis Biochemicals, Lausen, Switzerland). Blood was centrifuged at 3000 RPM and at a temperature of 4˚C for 15 minutes to isolate plasma. Plasma was separated and transferred to micro tubes for later analysis. Two micro tubes were pre-treated with hydrochloric acid (1N, 100microlitres per millilitre of plasma) to further protect acylated ghrelin from degradation. Plasma was stored at -70˚C until hormone assays were conducted. Acylated ghrelin, total PYY and total GLP-1 were measured in duplicate using ELISA (Human Ghrelin(active) ELISA kit, Millipore, MA, USA; Human PYY(total) ELISA kit, Millipore, MA, USA; Multi Species GLP-1(total) ELISA kit, MA, USA). The sensitivity of these ELISA kits were 8 $pg \bullet ml^{-1}$, 1.4 $pg \bullet ml^{-1}$ and 1.5 $pg \bullet ml^{-1}$ respectively and the coefficient of variation values were 2.36%, 5.26% and 3.28% respectively.

Statistical analysis

Data are presented as means ± standard deviation (SD) in tables and text and as mean ± standard error of the mean (SEM) in figures. For the determination of differences in energy intake from the test meal between each exercise condition, a one-way analysis of variance (ANOVA) with repeated measures was conducted. To compare differences in both subjective appetite and plasma concentration of appetite-associated hormones with time and between trial conditions, a 2-way factorial ANOVA with repeated measures was conducted. Post-hoc pairwise comparisons were conducted using the Bonferroni correction for multiple comparisons. For all analyses of variance, there were no significant between-condition differences at baseline. The Shapiro Wilks test for normality revealed that data for all outcome measures were normally distributed.

To further investigate the relationship between changes in appetite hormones and changes in subjective appetite with exercise, correlation analysis was conducted for within-subject comparisons. For data of REST, subjective appetite scores and hormone concentrations immediately prior to the *ad libitum* test meal were correlated with energy intake using Pearson product moment correlation. Hormone concentration was correlated with appetite scores immediately prior to the test meal, as well as at t=0. Data of all three exercise trials were collated and Pearson product moment correlation coefficients with repeated observations were calculated, as described by Bland & Altman (Bland & Altman, 1995). With this collated data, subjective appetite scores and hormone concentrations immediately prior to the *ad libitum* test meal were correlated with energy intake. Hormone concentrations were correlated with subjective appetite scores immediately prior to the test meal. To assess the relationship between changes in appetite-associated hormones and subjective appetite in the immediate post exercise period, absolute values at t=0 and change-from-baseline values at t=0 were correlated for hormone concentration and subjective appetite scores.

A statistical significance level of $p<0.05$ was used throughout. When significant differences were observed, effect sizes were calculated. For all analyses of variance (ANOVA), effect size was calculated as partial eta squared (η^2_p). For pairwise comparisons of note effect size was calculated as Cohen's d (d) and 95% confidence intervals (CI) are expressed. All statistical analysis was carried out using the SPSS software programme (SPSS inc.. Chicago, Illinois, USA).

An a priori power calculation was conducted using data from an unpublished study conducted within our laboratory (effect size $\eta^2_p=0.291$ from a repeated measures factorial ANOVA model. A. Holliday & A.K. Blannin). Attributing subjective appetite as the primary outcome measure, and using an alpha level of 0.05 and a statistical power of 0.8, the calculation yielded a required sample size of 12 participants. This sample is powered

to detect a medium effect (*f*=0.62 in the power calculation) which, based on the aforementioned unpublished data, represents minimum difference of 12% in subjective appetite.

RESULTS

Exercise trials

The characteristics of the four trial conditions are shown in T**able 2**. Absolute and relative intensity (VO_2 and $\%VO_{2max}$) did not differ between exercise trials. There was, however, a trend for a main effect of trial for mean power output (p=0.052). Rating of perceived exertion was significantly lower for 15-MIN than both 30-MIN and 45-MIN, with no difference between 30-MIN and 45-MIN. The energy expenditure of the exercise bouts differed significantly between the three exercise conditions (p<0.001), resulting in significant differences in total energy expenditure of the entire trial period between the four conditions (p<0.001).

(Table 2)

Subjective Appetite

VAS

Appetite profiles obtained using the VAS technique are shown in F**igure 1** for each condition. There was no condition x time interaction (p=0.083, η^2_p=0.163), nor condition main effect (p=0.244, η^2_p=0.119). There was a significant main effect for time (p<0.001, η^2_p=0.779), which showed that appetite rose from the cessation of exercise (t=0) until the test meal (t=60), before falling after feeding.

(Figure 1)

Food intake at the ad libitum test meal

The mean energy intake values for each of the four trial conditions are shown in **Figure 2a**. There was no condition effect for energy intake (p=0.223, η^2_p=0.130), suggesting that intakes were similar (REST = 3268±1397 kJ, 15-MIN = 3474±1233 kJ, 30-MIN = 3636±1254 kJ, 45-MIN = 3769±1591 kJ). When accounting for the energy expenditure of exercise and assessing relative energy intake (REI, intake–expenditure), there was a significant main effect for condition (p=0.003, η^2_p=0.573, **Figure 2b**). REI was significantly greater in REST (2641±1616 kJ) versus 30-MIN (1039±1520 kJ, p<0.001, d=1.03, 95% CI=908 –

2297 kJ) and 45-MIN (260±1731 kJ, p=0.001, d=1.42, 95% CI=1113 – 3648 kJ), while REI in 45-MIN was also significantly lower than 15-MIN (2699±1239 kJ, p=0.039, d=1.62, 95% CI=-4761 – -117 kJ). There were no significant differences in macronutrient content of the food consumed between the four conditions (**Table 3**).

(Figure 2)

(Table 3)

Plasma appetite-associated hormone concentrations

Acylated ghrelin

There was a significant condition x time interaction for acylated ghrelin concentration (p=0.011, η^2_p=0.285. **Figure 3**). Post-hoc analysis of between-condition comparisons showed that, immediately post-exercise (t=0), acylated ghrelin was significantly lower in 45-MIN (198±29 pg•mL^{-1}) versus REST (369±48 pg•mL^{-1}, p=0.009, d=1.307, 95% CI=-286 – -40 pg•mL^{-1}). Concentrations were also lower than REST in 15-MIN (273±42 pg•mL^{-1}) and 30=MIN (246±33 pg•mL^{-1}) immediately post-exercise, with these differences approaching statistical significance (p=0.077, d=0.910, 95% CI=-193 – 8 pg•mL^{-1} and p=0.055, d=0.971, 95% CI=-239 – 2 pg•mL^{-1}, respectively). The difference in acylated ghrelin concentration between 45-MIN and 30-MIN also approached significance (p=0.057, d=0.963, 95% CI=-89 – 1 pg•mL^{-1}). The difference in plasma ghrelin concentration between 45-MIN and REST remained significant at t=20 (239±35 pg•mL^{-1} vs. 365±47 pg•mL^{-1}, p=0.023, d=1.096, 95% CI=-203 – -14 pg•mL^{-1}). There were no significant differences between conditions at t=40 onwards.

Within-condition comparisons showed that acylated ghrelin concentration did not change, relative to baseline, in REST or 15-MIN. In the 30-MIN condition acylated ghrelin decreased during exercise, with a trend for lower concentration immediately post-exercise versus baseline (246±33 pg•mL^{-1} vs. 396±48 pg•mL^{-1} , p=0.098, d=1.093, 95% CI=-305 – 16 pg•mL^{-1}). This difference approached statistical significance at t=20 (249±7 pg•mL^{-1} vs. 396±8 pg•mL^{-1}, p=0.05, d=1.216, 95% CI=-283 – 0 pg•mL^{-1}). Mean acylated ghrelin concentration decreased to the greatest extent in the 45-MIN trial, with concentrations significantly lower immediately post-exercise (198±29 pg•mL^{-1}) and at t=20 (239±35 pg•mL^{-1}), versus baseline (366±47 pg•mL^{-1}, p=0.038, d=1.269, 95% CI=-312 – -7 pg•mL^{-1} and p=0.025, d=1.346, 95% CI=-207 – -10 pg•mL^{-1} respectively).

(Figure 3)

PYY

There was no significant condition x time interaction for PYY concentration ($p=0.472$, $\eta^2_p=0.080$), nor was there a significant main effect for condition ($p=0.691$, $\eta^2_p=0.252$) (**Figure 4**). A significant time main effect ($p<0.001$, $\eta^2_p=0.522$) demonstrated a decrease in PYY concentration during the post-exercise period, compared with baseline, until the test meal (t=60).

(Figure 4)

GLP-1

There was a significant time x condition interaction for GLP-1 plasma concentration ($p<0.001$ $\eta^2_p=0.433$; F**igure 5**). Post-hoc analysis of between-condition comparisons showed that, at t=0, there was a trend for a greater GLP-1 concentration in the 30-MIN trial (33.4±11.1 pg•mL^{-1}) compared with REST (26.5±10.0 pg•mL^{-1}, p=0.093, d=0.878, 95% CI=-0.9 – 14.7 pg•mL^{-1}) and a greater concentration in 45-MIN (38.5±19.2 pg•mL^{-1}) versus 15-MIN (28.4±13.8 pg•mL^{-1}, p=0.076, d=0.912, 95% CI=-0.7 – 18.2 pg•mL^{-1}). At t=20, the trend for a greater concentration in 30-MIN versus REST was maintained, while plasma GLP-1 concentration was significantly greater in 45-MIN (40.6±19.9) versus 15-MIN (27.1±13.8 pg•mL^{-1}, p=0.024, d=1.121, 95% CI=1.4 – 22.7 pg•mL^{-1}) and greater in 45-MIN (40.6±19.9) versus REST, with the difference approaching significance (vs. 26.7±10.1 pg•mL^{-1}, p=0.080, d=0.905, 95% CI=-1.1 – 25.9 pg•mL^{-1}). This elevated concentration in 45-MIN was significantly greater than both REST and 15-MIN at t=40 (p=0.035 and p=0.047 respectively) and t=60 (p=0.012 and p=0.040 respectively), remaining higher than REST immediately after the test meal (p=0.035). Plasma GLP-1 concentration was significantly higher in 30-MIN, compared with REST at t=60 (p=0.032).

Post-hoc analysis of within-condition differences showed that, in 30-MIN, plasma GLP-1 increased above baseline concentration at t=0 (33.4±11.1 vs. 25.9±9.0 pg•mL^{-1}, p=0.018, d=1.418, 95% CI=1.1 – 14.0 pg•mL^{-1}), remaining elevated until t=40. In 45-MIN, GLP-1 concentration increased significantly above baseline at t=0 (38.5±19.2 vs. 25.8±12.4 pg•mL^{-1}, p=0.043, d=1.243, 95% CI=0.2 – 22.4 pg•mL^{-1}) and stayed elevated for the remainder of the trial period.

(Figure 5)

Relationship between hormones, subjective appetite and food intake.

In REST, there were no significant correlations, or trends for correlations, between hormone concentration and subjective appetite scores, either at t=0 or immediately prior to the test meal (all $r < \left| 0.5 \right|$, $p>0.1$). In addition neither VAS score nor concentrations of acylated ghrelin, PYY or GLP-1 were significantly correlated with energy intake ($r < \left| 0.5 \right|$, $p>0.1$).

After exercise, there was a trend for a strong positive correlation between acylated ghrelin concentration and subjective appetite score at t=0 (r=0.665, p=0.087). However, there were no other significant relationships, or trends for relationships, between hormone concentration and subjective appetite scores, either at t=0, as change-from-baseline at t=0, or immediately prior to the test meal (all $r < \left| 0.6 \right|$, $p>0.1$). PYY concentration immediately prior to the test meal showed a moderate, negative correlation with energy intake (r=-0.484, p=0.019) and with relative energy intake (r=-0.417, p=0.048). GLP concentration immediately prior to the test meal showed a moderate, negative correlation with relative energy intake (r=-0.599, p=0.002), but was not significantly associated with absolute energy intake. Acylated ghrelin concentration was associated with neither absolute nor relative energy intake.

DISCUSSION

The aim of the current study was to assess the effect of the duration of high-intensity aerobic exercise on subjective appetite, food intake and appetite-regulating hormones in highly-trained male endurance athletes. Subjective appetite was not significantly suppressed post-exercise in any of the three exercise conditions, with only modest, non-significant reductions of ~10-15% in appetite scores from baseline, and scores in none of the three exercise conditions differed significantly from the resting condition scores at any point in the trial period. This was despite significant responses of both acylated ghrelin and GLP-1 towards a more anorexigenic state.

Exercise at an intensity ≥60% VO_{2max} often elicits a transient suppression of appetite in untrained, lean individuals (Martins *et al*., 2007, King et al., 2010, Laan *et al.*, 2010; Deighton *et al.*, 2013; Kawano *et al*., 2013). To the knowledge of the authors, the exercise of the present study is the highest intensity bout of continuous exercise utilised in research of this nature. Yet, no suppression of appetite was observed. It is possible that this lack of a commonly-observed appetite suppression is due to a difference in responses to

exercise between athletic and non-athletic populations. Though equivocal, studies conducted in recreationally active young males with mean VO_{2max} values of ~55-57 $mL \bullet kg^{-1} \bullet min^{-1}$, utilising prolonged bouts of continuous exercise at ~65-70% VO_{2max} have elicited modest, non-significant appetite suppression similar to those of the present study (King *et al*., 2011; Wasse *et al*., 2013; Deighton *et al.,* 2014). These data, allied with the findings of the current study, suggest that appetite response to high-intensity aerobic exercise may be somewhat different for individuals of differing training/activity status or fitness. However, in contrast to the findings of the current study, Howe and colleagues (Howe *et al*., 2016) did observe a suppression of appetite after both moderate- (60% VO_{2max}) and high-intensity (80% VO_{2max}) in trained females. The differing responses could be due to the different sex of participants (although there is currently minimal evidence to suggest sex differences in acute appetite responses to exercise in non-athletes (Thackray *et al.*, 2016)). A limitation of the present study is the lack of a female study cohort for the direct investigation of sex differences. Nonetheless, the present findings suggest that regular endurance exercise training may blunt the appetite suppression response typically observed in those less familiar with such exercise.

Aligned with the absence of a post-exercise appetite suppression, there was no significant difference in food intake at the *ad libitum* test meal, administered 60 minutes after the cessation of exercise. No difference in food intake post-exercise is a commonly observed finding, especially when the test meal is consumed 60 minutes post-exercise (Thompson et al., 1988, King et al., 1997, King et al., 2010, King et al., 2011b, Schubert et al., 2013). The absence of a reduction in food intake after exercise would indicate, in contrast to the hypothesis, that the prolonged bout of continuous, high-intensity exercise of the present study was not sufficient to induce an appetite suppression that was sufficiently enduring to influence food intake 60 minutes post-exercise. It is acknowledged that a limitation of the present study is the acute measure of food intake. There is evidence that compensatory increases in food intake can occur in the hours and days after exercise, and that this response may differ depending on physical activity status (Rocha *et al.*, 2013). Therefore, monitoring food intake over the remainder of the trial day and perhaps over the following 48hours would have proved insightful.

Relative energy intake did differ between trial conditions. This has often been observed in the absence of a lower post-exercise absolute energy intake (Imbeault *et al.*, 1997, Lluch *et al.*, 2000, King et al., 2010, Unick *et al.*, 2010), or even after absolute energy intake is greater post-exercise (Pomerleau *et al.*, 2004, Martins et al., 2007). As such, it would appear there was a lack of an immediate compensatory increase in drive to eat after prolonged strenuous exercise and extensive energy expenditure, and that such exercise can elicit short-term energy deficit, compared with rest. However, the monitoring of food intake, physical activity and metabolic rate

over the course of the whole day and beyond would be required to determine likely effects of the exercise bout, and subsequent appetite response, on energy balance.

The post-exercise period is important for athletes with regard to nutrition for recovery and adaptation to training. Post-exercise carbohydrate intake is valued by many endurance athletes, with exercise-induced GLUT-4 translocation leading to an increased potential for glucose uptake and glycogen resynthesis after exercise (see Ivy, 1998, Goodyear *et al*., 1998, Jentjens & Jeukendrup, 2003). In addition, amino acid delivery and a positive energy balance stimulate net muscle protein synthesis after resistance- (Tipton *et al*., 1999) and endurance-type exercise (Howarth *et al*., 2009), meaning that the ingestion of protein in close proximity to exercise is often desired for optimal rates of muscle protein synthesis and subsequent adaptation (Phillips, 2006, Phillips & Van Loon, 2011). Therefore, a suppression of appetite post-exercise may be detrimental for recovery and adaptation, should it impact upon nutrition. The findings of the present study would suggest that this was not the case, even after a strenuous bout of 45 minutes of cycling at 76% VO_{2max}.

A lack of significant exercise-induced suppression of appetite in the present study was allied with no significant change in plasma concentration of the satiety peptide PYY with exercise. While PYY concentration has commonly been observed to be responsive to high-intensity aerobic exercise (Martins *et al*., 2007, Broom *et al*., 2009, Russel *et al.*, 2009, Ueda *et al*., 2009b, Ueda *et al.*, 2009a, Larson-Meyer *et al.*, 2012), this was not the case in the present study. It is possible that this is due to the study population; well-trained athletes, familiar with exercising at such a high-intensity may be resistant to exercise-induced alterations in PYY secretion. Chronic exercise training has been postulated to sensitise satiety peptides to food intake, with greater late post-prandial period concentrations of PYY and GLP-1 with food intake after exercise training (Martins *et al.*, 2010). In addition, exercise training (Jones *et al.*, 2009) and exercise-induced weight-loss (Roth *et al.*, 2005) have been shown to increase fasting PYY concentrations. These may be mechanisms by which regular physical activity assists with tighter regulation of energy balance, through limiting over-eating. Similarly, the blunting of an exercise-induced increase in PYY with regular exercise may regulate energy balance through the avoidance of appetite suppression and increased energy deficit post-exercise. A limitation of the present study is that concentrations of total PYY were measured, rather than the active form $PYY_{(3-36).}$ However, the abundance of $PYY_{(3-36)}$ is greater than that of $PYY_{(1-36)}$, meaning that most of the total circulating PYY in plasma is in the form of $PYY_{(3-36).}$ Additionally, total PYY and $PYY_{(3-36)}$ have been shown to respond similarly to food intake (Pfluger *et al*., 2007), and have demonstrated similar responses to exercise of a similar nature (Deighton *et al*., 2013a and 2013b). Hence, responses of total PYY to exercise are likely to reflect those of $PYY_{(3-36).}$

Despite no significant suppression of appetite, there was a clear response of GLP-1 during and after exercise. Concentrations rose with exercise in the 30-MIN (29%) and 45-MIN (49%) conditions, with levels remaining elevated during the 60 minute recovery period. This finding is in agreement with previous studies in obese (Ueda *et al*., 2009b) and healthy-weight (Martins *et al*., 2007, Ueda *et al*., 2009a) individuals, after exercise of an intensity lower than that of the current study, lasting 30-60 minutes. No such response was observed in the 15-MIN condition however, with the GLP-1 profile closely resembling that of REST. This is in contrast to previous studies that have observed a suppression of GLP-1 with very low volume, high-intensity interval exercise in recreationally active (Bailey *et al.*, 2015) and overweight (Martins *et al*., 2015) individuals. These data would suggest that just 15 minutes of high-intensity cycling at 76% VO_{2max} was an insufficient stimulus to cause any exercise-induced increase in plasma GLP-1 in trained endurance athletes, and that the contrast with observations from maximal, or near maximal, high-intensity interval exercise studies could be due to the differing intensity of exercise or differing study populations. These data would also suggest that GLP-1 concentration during high-intensity aerobic exercise exhibits some duration or energy expenditure dependency, possibly with a threshold duration for its secretion.

Acylated ghrelin also proved responsive to exercise. The plasma concentration declined with exercise in all three exercise conditions, with the greatest decrease seen after 45 minutes of cycling. This suppression of acylated ghrelin was transient, with concentrations not significantly different to baseline by 40 minutes post-exercise, even in 45-MIN; neither was there a significant difference between any exercise condition and REST at this time point. This was despite acylated ghrelin concentration being 28%, 20% and 23% lower than REST in the 15-MIN, 30-MIN and 45-MIN conditions, respectively. As with the finding of Broom et al (2017), this present study would indicate that acylated ghrelin responses to exercise may be duration-dependent. However, while the findings of Broom et al. (2017) suggest duration-dependent differences in the longevity of the suppression, the present findings suggest difference in the magnitude of the suppression. Data of the present study would suggest that, with high-intensity aerobic exercise, plasma acylated ghrelin concentration begins to decline in the very early stages of exercise and continues to decline as the bout continues. While this would suggest a physiological mechanism by which the duration of exercise is an important regulatory factor in post-exercise appetite suppression, the absence of a significant suppression of appetite (either compared with baseline or with REST) dispels this theory somewhat and also questions the role of acylated ghrelin as a regulator of appetite in the post-exercise state.

It would appear there are some inconsistencies in the hormonal response and appetite in the present study. Firstly, changes in acylated ghrelin and GLP-1 in favour of an anorexigenic state were not observed with PYY. It has generally been observed that alterations in appetite-associated hormones occur concurrently, especially with regards to satiety peptides (Broom *et al*., 2009, King *et al.*, 2011a). Differential responses in PYY and GLP-1 have, however, been observed following 30 minutes of cycling at 50% VO_{2max} and 75% VO_{2max} (Ueda *et al*., 2009a). It was found that PYY secretion appeared to be exercise intensity-dependent, with concentration elevated to a greater extent after exercise at 70% VO_{2max}, compared with after exercise at 50% VO_{2max}. In contrast, GLP-1 concentration increased similarly in both exercise trials. The authors suggest that their data would advocate specific exercise responses in plasma kinetics of PYY and GLP-1. The data of the present study would support the notion of a specific response, but contrasts the findings somewhat, with GLP-1, but not PYY, suggested to change in a duration- or energy expenditure-dependant manner. Further, if an increase in plasma PYY is exercise intensity-dependent, then it may be the case that athletes possess a blunted response, or have elevated their threshold intensity for PYY release.

Secondly, the anorexigenic stimulus of an increase in GLP-1 concentration and a decrease in acylated ghrelin was not reflected by a suppression of subjective appetite or reduced absolute food intake. Both total (Wren *et al*., 2001) and acylated ghrelin (Druce *et al.*, 2005), have been shown to be potent appetite regulators when administered pharmaceutically in the resting state. However, some studies infused non-physiological concentrations (Wren *et al*., 2001), while lower concentration infusion has yielded conflicting effects on food intake on overweight and lean individuals (Druce *et al*., 2005). Studies investigating the effect of GLP-1 administration, at a physiological concentration, on food intake are equivocal (Verdich *et al*., 2001). In the present study, exercise-induced alterations that would be expected to favour an anorexigenic state did not lead to a suppression of subjective appetite in the post-exercise period.

Assessment of the relationships for within-subject changes in appetite, hormone concentration and energy showed little consistent association between concentration of hormones and subjective appetite, both at rest and post-exercise. There was a trend for a strong correlation between acylated ghrelin and VAS score immediately post-exercise, which does suggest that immediate post-exercise appetite responses may be mediated by changes in acylated ghrelin. However, this association was not statistically significant and was not evident at other post-exercise measures. Neither PYY nor GLP-1 were associated with subjective appetite at any time. However, PYY concentration immediately prior to the test meal was inversely related to energy intake, and both PYY and GLP-1 concentration were inversely related to relative energy intake. Such inconsistencies

are not uncommon (Broom *et al.*, 2007, Broom *et al*., 2009) and there is evidence that in the post-exercise period, there is blunting to hormonal regulators of appetite. In a study by Heden *et al.* (Heden *et al.*, 2013), acylated ghrelin and subjective appetite responded differently with exercise in healthy-weight and obese individuals, and Deighton *et al.* (Deighton *et al.*, 2013) observed contrasting positive and negative correlations between acylated ghrelin and subjective appetite in the period after endurance and sprint-interval exercise, respectively, in healthy-weight males. Further, previous studies have also shown weak (Broom *et al*., 2009; Hagobian *et al*., 2013; Wasse *et al*., 2013; Beaulieu *et al*., 2014) or inconsistent (Ueda *et al*., 2009a; Deighton *et al*., 2014; Bailey *et al*., 2015) relationships between hormone concentration and both subjective appetite and food intake, yet the relevance of such findings are largely overlooked. These data question the commonly-accepted importance of exercise-induced changes in appetite-associated hormones for appetite regulation and acute absolute energy intake. Although, the data of the present study suggests that the satiety peptides PYY and GLP-1 may influence relative energy intake. As such, it is possible that the role of these hormones is to defend against overeating and a compensation for energy expenditure, as opposed to suppressing food intake *per se*. Further investigation is required to clarify the regulatory role of these hormones, at physiological concentrations, in appetite and food intake responses, especially in the post-exercise period.

In conclusion, neither 15, 30 nor 45 minutes of cycling at 76% VO_{2max} significantly suppressed subjective appetite in male highly-trained endurance athletes. Acute absolute food intake was unaffected by exercise, although with no compensatory increase in energy intake, exercise of 30 minutes and 45 minutes in duration induced an acute energy deficit, compared with remaining rested. The lack of observed appetite suppression was despite a transient suppression of acylated ghrelin and a sustained increase in GLP-1, with some evidence that the concentration of these hormones change in an exercise-duration-dependent manner. These findings suggest that those accustomed to high-intensity aerobic exercise may exhibit a blunted response to exercise-induced appetite suppression, or a dissociation of appetite perception and hormonal signals post-exercise. The role of appetite-associated hormones in regulating post-exercise appetite, food intake and acute energy balance warrants further investigation.

DECLARATION OF INTERESTS

The authors have no competing interests to declare.

FUNDING

Adrian Holliday was funded by a University of Birmingham PhD Studentship

ACKNOWLEDGEMENTS

The authors would like to acknowledge and thank Dr Oliver Wilson for assisting with the drafting of the manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

ETHICAL APPROVAL

Ethical approval was obtained from the Ethics Subcommittee of the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham. Ethical Review Number – ERN_09-996. All research was performed in accordance with the 1964 Declaration of Helsinki.

ETHICS, CONSNET AND PERMISSION

Informed written consent was obtained from each participant after both written and verbal information about the study was provided. This consent included permission to publish research data.

CONSENT TO PUBLISH

Informed written consent was obtained from each participant after both written and verbal information about the study was provided. This consent included permission to publish research data. No personal information of any participant is included in the manuscript.

AVAILABILITY OF DATA

The raw data is available as a supplementary article to the manuscript.

AUTHOR CONTRIBUTIONS

AH and AB conceived the study question and study design; AH completed the data collection and data analysis; AH wrote the manuscript; AB assisted with the drafting of the manuscript.

REFERENCES

Bailey DP, Smith LR, Chrismas BC, Taylor L, Stensel DJ, Deighton K, Douglas JA & Kerr CJ 2015 Appetite and gut hormone responses to moderate-intensity continuous exercise versus high-intensity interval exercise, in normoxic and hypoxic conditions. *Appetite* **89** 237-245.

Bellissimo N, Thomas SG, Goode RC & Anderson GH 2007 Effect of short-duration physical activity and ventilation threshold on subjective appetite and short-term energy intake in boys. *Appetite* **49** 644-651.

Bland JM & Altman DG 1995 Statistics notes: Calculating correlation coefficients with repeated observations: Part 1—correlation within subjects. *British Medical Journal* **310** 446.

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

Broom, DR, Miyashita M, Wassw, LM, Pulsford R, King JA, Thackray AE & Stensel DJ 2017 Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *Journal of Endocrinology* **232** 411-422.

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

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** 1-10.

Deighton K, Batterham RL & Stensel DJ 2014 Appetite and gut hormone responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite* **81**(1) 52-59.

Deighton, K., J. C. Zahra and D. J. Stensel, 2012 Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state. *Appetite,* **58,** 946-954.

Druce MR, Neary NM, Small CJ, Milton J, Monteiro M, Patterson M, Ghatei MA & Bloom SR 2005 Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *International Journal of Obesity and Related Metabolic Disorders* **30** 293-296.

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.

Filaire E, Rouveix M, Pannafieux C, Ferrand C. 2007 Eating Attitudes, Perfectionism and Body-esteem of Elite Male Judoists and Cyclists. *Journal of Sports Science & Medicine* **6**(1) 50-7.

Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. Annual Review of Medicine. 1998;49(1):235-61. doi:doi:10.1146/annurev.med.49.1.235

Hagobian TA, Yamashiro M, Hinkel-Lipsker J, Streder K, Evero N & Hackney T 2013 Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Applied Physiology Nutrition and Metabolism* **38** 66-72

Heden TD, Liu Y, Park Y, Dellsperger KC & Kanaley JA 2013 Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology* **115** 680-687.

Hill AJ & Blundell JE 1982 Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research* **17** 203-212.

Holliday A & Blannin K 2017 Very low volume sprint interval exercise suppresses subjective appetite, lowers acylated ghrelin, and elevates GLP-1 in overweight individuals: A pilot study. *Nutrients* **9** 362-376

Howarth KR, Moreau NA, Phillips SM, Gibala MJ. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. Journal of Applied Physiology. 2009;106(4):1394-402. doi:10.1152/japplphysiol.90333.2008.

Howe S, Hand T, Larson-Meyer D, Austin K, Alexander B & Manore M 2016 No Effect of Exercise Intensity on Appetite in Highly-Trained Endurance Women. *Nutrients* **8** 223.

Imbeault P, Saint-Pierre S, Alm-Ras N & Tremblay A 1997 Acute effects of exercise on energy intake and feeding behaviour. *British Journal of Nutrition* **77** 511-521.

Ivy JL. Glycogen resynthesis after exercise: effect of carbohydrate intake. International Journal of Sports Medicine. 1998;19 Suppl 2:S142-5.

Jentjens R, Jeukendrup A. Determinants of Post-Exercise Glycogen Synthesis During Short-Term Recovery. Sports Medicine. 2003;33(2):117-44. doi:10.2165/00007256-200333020-00004.

Jones TE, Basilio JL, Brophy PM, McCammon MR & Hickner RC 2009 Long-term Exercise Training in Overweight Adolescents Improves Plasma Peptide YY and Resistin. *Obesity* **17** 1189-1195.

Jurimae J, Hofmann P, Jurimae T, Maestu J, Purge P, Wonisch M et al. 2006 Plasma Adiponectin Response to Sculling Exercise at Individual Anaerobic Threshold in College Level Male Rowers. *International Journal of Sports Medicine* **27**(04) 272-277.

Jurimae J & Jurimae T 2005 Leptin responses to short term exercise in college level male rowers. *British Journal of Sports Medicine* **39**(1) 6-9. doi:10.1136/bjsm.2003.008516.

Jurimae J, Jurimae T & Purge P 2007 Plasma Ghrelin Is Altered After Maximal Exercise in Elite Male Rowers. *Experimental Biology and Medicine* **232**(7):904-9.

Jürimäe J, Mäestu J & Jürimäe T 2003 Leptin as a marker of training stress in highly trained male rowers? *European Journal of Applied Physiology*. **90** 533-538. doi:10.1007/s00421-003-0879-2.

Jurimae J, Ramson R, Maestu J, Purge P, Jurimae T, Arciero PJ et al. 2009 Plasma Visfatin and Ghrelin Response to Prolonged Sculling in Competitive Male Rowers. *Medicine & Science in Sports & Exercise*. **41**(1) 137-143 10.1249/MSS.0b013e31818313e6.

Kawano H, Mineta M, Asaka M, Miyashita M, Numao S, Gando Y, Ando T, Sakamoto S & Higuchi M 2013 Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* **66** 26-33.

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

King JA, Wasse LK, Ewens J, Crystallis K, Emmanuel J, Batterham RL & Stensel DJ 2011a Differential Acylated Ghrelin, Peptide YY3-36, Appetite, and Food Intake Responses to Equivalent Energy Deficits Created by Exercise and Food Restriction. *Journal of Clinical Endocrinology & Metabolism* **96** 1114-1121.

King JA, Wasse LK & Stensel DJ 2011b The Acute Effects of Swimming on Appetite, Food Intake, and Plasma Acylated Ghrelin. *Journal of Obesity* **2011**.

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

King NA, Tremblay A & Blundell JE 1997 Effects of exercise on appetite control: implications for energy balance. *Medicine & Science in Sports & Exercise* **29** 1076-1089.

Kissileff HR, Pi-Sunyer FX, Segal K, Meltzer S & Foelsch PA 1990 Acute effects of exercise on food intake in obese and nonobese women. *American Journal of Clinical Nutrition* **52** 240-245.

Laan DJ, Leidy HJ, Lim E & Campbell WW 2010 Effects and reproducibility of aerobic and resistance exercise on appetite and energy intake in young, physically active adults. *Applied Physiology, Nutrition, and Metabolism* **35** 842-847.

689 Larson-Meyer D, Palm S, Bansal A, Austin KJ, Hart AM & Alexander BM 2012 Influence of Running and
690 Walking on Hormonal Regulators of Appetite in Women. *Journal of Obesity* **2012** 15.
691
692 Lluch A, King NA & Blundell JE 2000 No energy compensation at the meal following exercise indietary
693 restrained and unrestrained women. *British Journal of Nutrition* **84** 219-225.
694
695 Martins C, Kulseng B, King NA, Holst JJ & Blundell JE 2010 The Effects of Exercise-Induced Weight Loss on
696 Appetite-Related Peptides and Motivation to Eat. *Journal of Clinical Endocrinology & Metabolism* **95** 1609-
697 1616.
698
699 Martins C, Morgan LM, Bloom SR & Robertson MD 2007 Effects of exercise on gut peptides, energy intake
700 and appetite. *Journal of Endocrinology* **193** 251-258.
701
702 Martins C, Stensvold D, Finlayson G, Holst J, Wisloff U, Kulseng B, Morgan L & King NA 2015 Effect of
703 moderate- and high-intensity acute exercise on appetite in obese individuals. *Medicine & Science in Sports &*
704 *Exercise* **47** 40-48.
705
706 O'Connor AM, Johnston CF, Buchanan KD, Boreham C, Trinick TR & Riddoch CJ 1995 Circulating
707 gastrointestinal hormone changes in marathon running. *International Journal of Sports Medicine* **16**(5) 283-287.
708
709 O'Connor AM, Pola S, Ward BM, Fillmore D, Buchanan KD & Kirwan JP 2006 The gastroenteroinsular
710 response to glucose ingestion during postexercise recovery. *American Journal of Physiology - Endocrinology*
711 *and Metabolism* **290**(6) E1155-61. doi:10.1152/ajpendo.00500.2005.
712
713 Phillips SM 2006 Dietary protein for athletes: from requirements to metabolic advantage. *Applied Physiology,*
714 *Nutrition, and Metabolism* **31**(6) 647-654. doi:10.1139/h06-035.
715
716 Phillips SM &Van Loon LJC 2011 Dietary protein for athletes: From requirements to optimum adaptation.
717 *Journal of Sports Sciences* **29**(sup1) S29-S38. doi:10.1080/02640414.2011.619204.
718
719 Pomerleau M, Imbeault P, Parker T & Doucet E 2004 Effects of exercise intensity on food intake and appetite in
720 women. *American Journal of Clinical Nutrition* **80** 1230-1236.
721
722 Rocha J, Paxman J, Dalton C, Winter E & Broom D 2013 Effects of an acute bout of aerobic exercise on
723 immediate and subsequent three-day food intake and energy expenditure in active and inactive men. *Appetite* **71**
724 369-378.
725
726 Roth CL, Enriori PJ, Harz K, Woelfle J, Cowley MA & Reinehr T 2005 Peptide YY Is a Regulator of Energy
727 Homeostasis in Obese Children before and after Weight Loss. *Journal of Clinical Endocrinology and*
728 *Metabolism* **90** 6386-6391.
729
730 Russel RR, Willis KS, Ravussin E & Larson-Meyer ED 2009 Effects of endurance running and dietary fat on
731 circulating ghrelin and peptide YY. *Journal of Sports Science & Medicine* **8** 574-583.
732
733 Schubert MM, Desbrow B, Sabapathy S & Leveritt M 2013 Acute exercise and subsequent energy intake. A
734 meta-analysis. *Appetite* **63** 92-104.
735
736 Schubert MM, Sabapathy S, Leveritt M & Desbrow B 2014 Acute Exercise and Hormones Related to Appetite
737 Regulation: A Meta-Analysis. *Sports Medicine* **44** 387-403.
738
739 Sundgot-Borgen J, Meyer NL, Lohman TG, Ackland TR, Maughan RJ, Stewart AD et al. How to minimise the
740 health risks to athletes who compete in weight-sensitive sports review and position statement on behalf of the
741 Ad Hoc Research Working Group on Body Composition, Health and Performance, under the auspices of the
742 IOC Medical Commission. *British Journal of Sports Medicine*. 2013;47(16):1012-22. doi:10.1136/bjsports-
743 2013-092966.
744
745 Thackray A, Deighton K, King JA & Stensel DJ, 2016 Exercise, Appetite and Weight Control: Are There
746 Differences between Men and Women? *Nutrients* **8** 583.
747

748 Thompson DA, Wolfe LA & Eikelboom R 1988 Acute effects of exercise intensity on appetite in young men.
749 *Medicine & Science in Sports & Exercise* **20** 222-227.
750
751 Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR 1999 Postexercise net protein synthesis in human
752 muscle from orally administered amino acids. *American Journal of Physiology - Endocrinology and Metabolism*
753 **276**(4):E628-E34.
754
755 Ueda S.-y, Yoshikawa T, Katsura Y, Usui T & Fujimoto S 2009a Comparable effects of moderate intensity
756 exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *Journal of*
757 *Endocrinology* **203** 357-364.
758
759 Ueda S.-y, Yoshikawa T, Katsura Y, Usui T, Nakao H & Fujimoto S 2009b Changes in gut hormone levels and
760 negative energy balance during aerobic exercise in obese young males. *Journal of Endocrinology* **201** 151-159.
761
762 Unick JL, Otto AD, Goodpaster BH, Helsel DL, Pellegrini CA & Jakicic JM 2010 Acute effect of walking on
763 energy intake in overweight/obese women. *Appetite* **55** 413-419.
764
765 van Strien T, Frijters JER, Bergers GPA & Defares PB 1986 The Dutch Eating Behavior Questionnaire (DEBQ)
766 for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders*
767 **5** 295-315.
768
769 Verdich C, Flint A, Gutzwiller J-P, Naslund E, Beglinger C, Hellstrom PM, Long SJ, Morgan LM, Holst JJ &
770 Astrup A 2001 A Meta-Analysis of the Effect of Glucagon-Like Peptide-1 (7-36) Amide on Ad Libitum Energy
771 Intake in Humans. *Journal of Clinical Endocrinology and Metabolism* **86** 4382-4389.
772
773 Wasse LK, Sunderland C, King JA, Miyashita M & Stensel DJ 2013 The influence of vigorous running and
774 cycling exercise on hunger perceptions and plasma acylated ghrelin concentrations in lean young men. *Applied*
775 *Physiology, Nutrition & Metabolism* **38** 106.
776
777 Westerterp-Plantenga MS, Verwegen CR, Ijedema MJ, Wijckmans NE & Saris WH 1997 Acute effects of
778 exercise or sauna on appetite in obese and nonobese men. *Physiology & Behavior* **62** 1345-1354.

779 Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA & Bloom SR 2001
780 Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and*
781 *Metabolism* **86**(12) 5992-

782

783

784

785

786

787

788

789

790

791

792

793

Table 1. Participant characteristics. Values are mean ± SD.

Age (years)	21 ± 2
Height (cm)	179.3 ± 7.2
Weight (kg)	67.3 ± 5.2
BMI ($kg \cdot m^{-2}$)	21.0 ± 1.6
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	61.6 ± 6.0
W_{max} (Watts)	309 ± 45
DEBQ score for restraint	1.9 ± 0.4

VO_{2max} = maximal aerobic capacity; RPE = rating of perceived exertion; W_{max} = maximal work load.

Table 2. Characteristics of exercise. Values are mean ± SD.

	REST	**15 min**	**30 min**	**45 min**
VO_2 ($mL \cdot min^{-1}$)	341 ± 33*	3150 ± 368	3180 ± 405	3138 ± 416
% VO_{2max}	6 ± 4*	76 ± 8	77 ± 8	76 ± 8
Power output (W)	-	218 ± 30†	207 ± 30	207 ± 33
% W_{max}	-	70 ± 4†	66 ± 6	66 ± 4
Heart rate ($beats\ min^{-1}$)	-	153 ± 13	156 ± 15	157 ± 14
% HR_{max}	-	84 ± 5	84 ± 5	86 ± 3
RPE	-	13 ± 1#	14 ± 1	15 ± 2
Energy Expenditure of bout (kJ)	156 ± 95*	989 ± 111*	1987 ± 252*	2929 ± 381*
EE of trial (bout + rec. period. kJ)	623 ± 52*	1420 ± 110*	2516 ± 157*	3414 ± 228*

***** significantly different to all other conditions, $p < 0.001$. † significantly different to 45 min, $p < 0.05$. # significantly different to 30 min and 45 min, $p < 0.05$.
VO_{2max} = maximal aerobic capacity; RPE = rating of perceived exertion; W_{max} = maximal work load.

826 **Table 3**. Summary of food intake at the *ad libitum* test meal for each of the four conditions. Values
827 are mean ± SD.
828

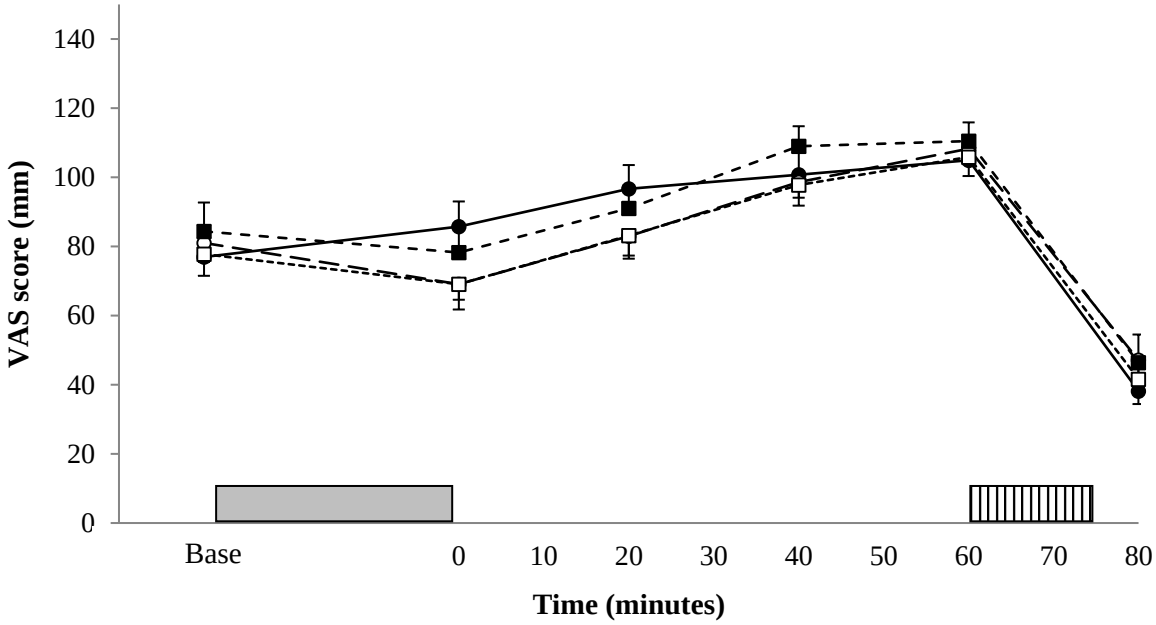
	REST	15-MIN	30-MIN	45-MIN
Weight consumed (grams)	735 ± 331	793 ± 281	836 ± 262	822 ± 264
Carbohydrate (g)	148 ± 64	157 ± 55	167 ± 55	165 ± 57
% energy CHO	76.3 ± 6.7	77.2 ± 5.7	77.5 ± 5.7	75.4 ± 9.3
Fat (g)	11.1 ± 6.6	11.3 ± 6.4	11.5 ± 6.4	17.2 ± 21.2
% energy fat	13.4 ± 5.8	12.9 ± 4.8	12.5 ± 4.7	15.3 ± 10.6
Protein (g)	20.6 ± 9.4	20.9 ± 8.2	22.3 ± 9.2	20.3 ± 8.3
% energy protein	10.3 ± 2.2	9.9 ± 2.0	10.0 ± 2.1	9.3 ± 2.3

829

830

831

832 **Figure 1**



833

834 **Figure 1**. Mean appetite scores, as measured using VAS. ●, solid line = REST; ○, large dash = 15-MIN; ■,
835 medium dash = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad*
836 *libitum* breakfast meal. Values are mean ± SEM.
837

838

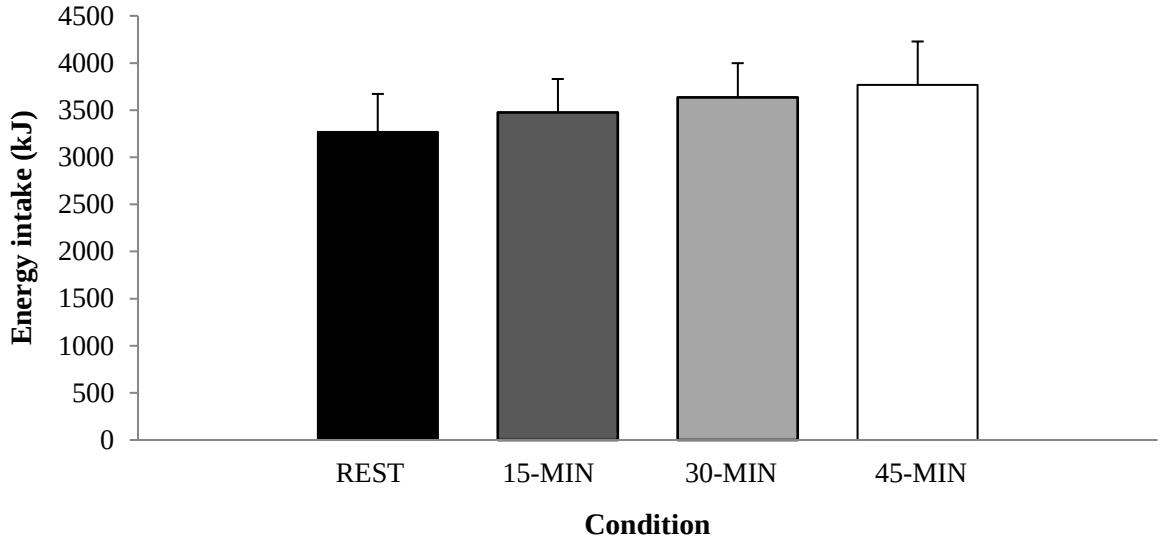
839

840

841

Figure 2

(a)



(b)

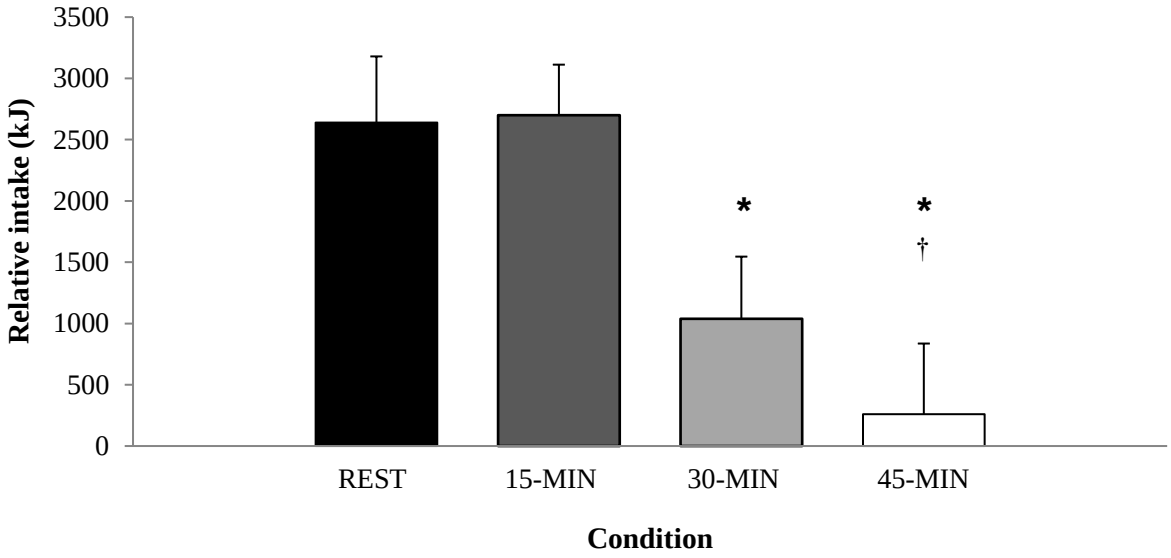


Figure 2. Energy intake (a) and relative energy intake (b) at the *ad libitum* breakfast test meal for REST, 15-MIN, 30-MIN and 45-MIN. Values are mean ± SEM. ***** significantly lower than REST. † significantly lower than 15-MIN.

Figure 3

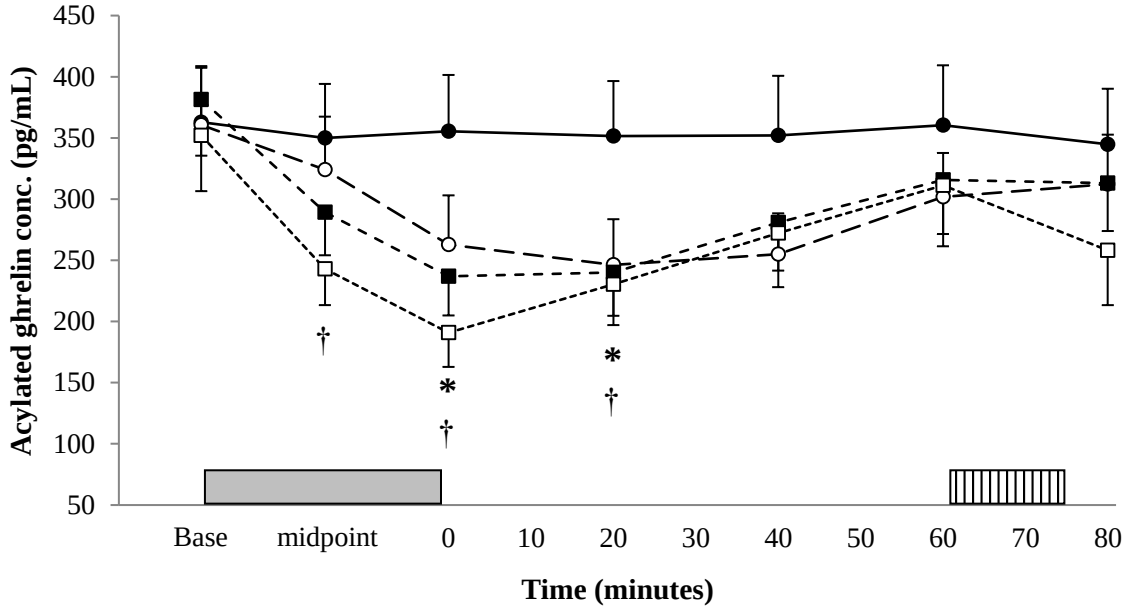


Figure 3. Mean plasma concentration of acylated ghrelin. ●, solid line = REST; ○, large dash = 15-MIN; ■, medium dash = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean ± SEM. ***** significant within-condition, lower than baseline. † significant between-condition effect, 45-MIN lower than REST.

Figure 4

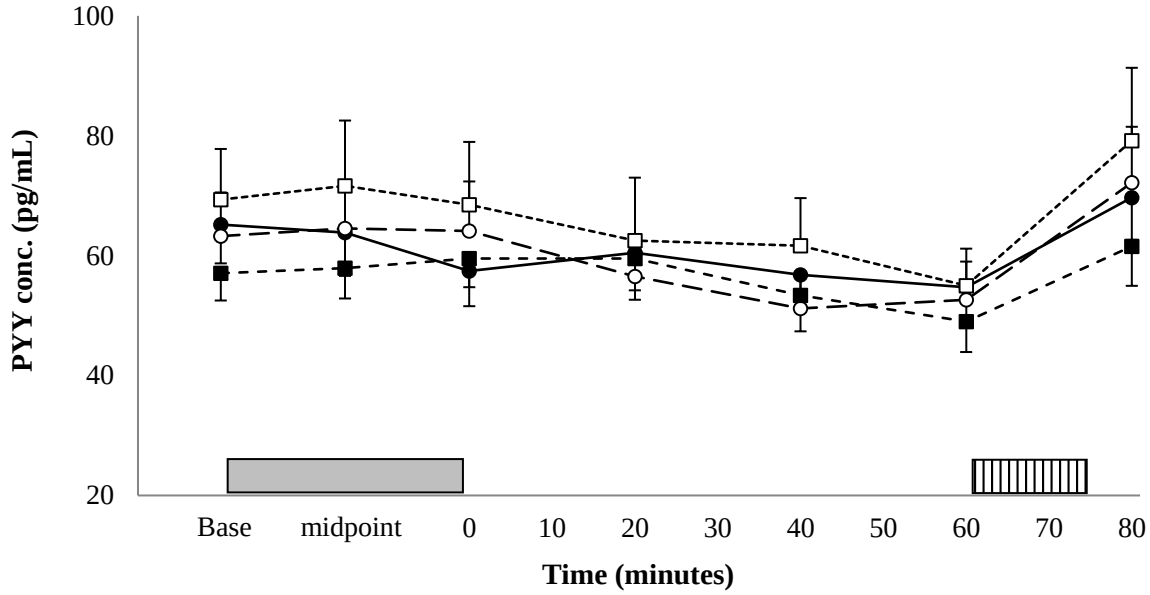
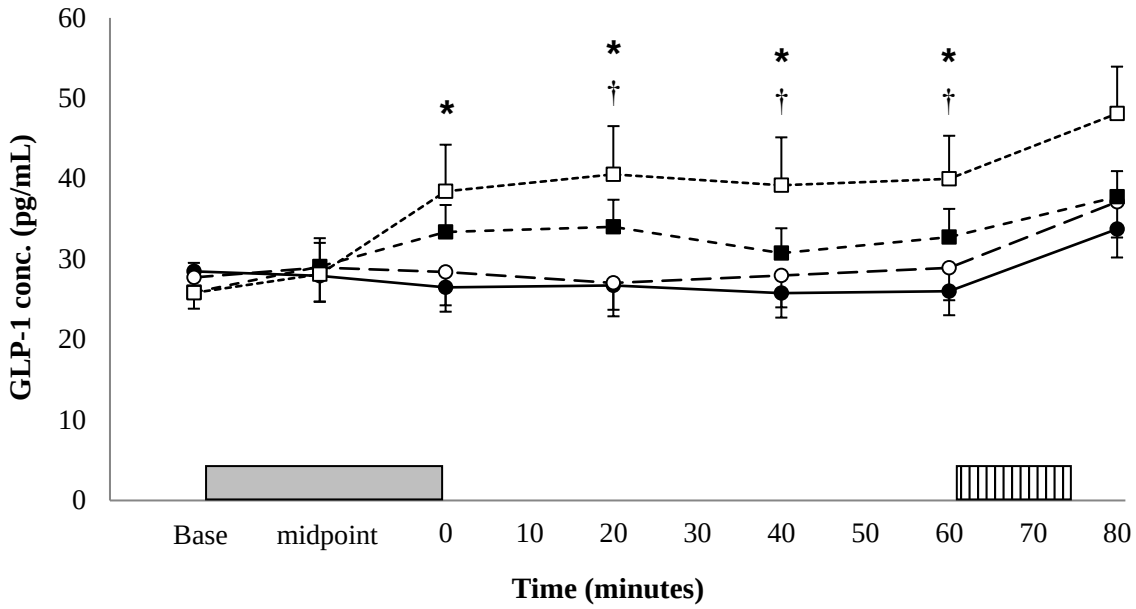


Figure 4. Mean plasma concentration of PYY. ●, solid line = REST; ○, large dash = 15-MIN; ■, medium dash = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast meal. Values are mean ± SEM.

870 **Figure 5**



871

872 **Figure 5**. Mean plasma concentration of GLP-1. ●, solid line = REST; ○, large dash = 15-MIN; ■, medium dash
873 = 30-MIN; □, small dash = 45-MIN. Hollow rectangle = exercise; filled, black rectangle = *ad libitum* breakfast
874 meal. Values are mean ± SEM. ***** significant within-condition effect, vs. baseline. † significant between-
875 condition effect.
876

877

878

879

880

881

882

883