# Appetite and gut peptide responses to exercise and calorie restriction: the effect of modest energy deficits.

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**Abstract**

Weight loss is the result of a sustained negative energy balance, which is typically achieved by decreasing food intake and/or increasing physical activity. Current evidence suggests that acute energy deficits of ~4820kJ elicit contrasting homeostatic responses when induced by exercise and food restriction but the response to government-recommended energy deficits is unknown. Twelve healthy men (mean(SD): age 24(5)years, body mass index 23.8(2.7)kg.m$^{-2}$, maximum oxygen uptake 55.4(9.1)mL.kg$^{-1}$.min$^{-1}$) completed three 8h trials (control (Con), exercise-induced energy deficit (Ex-Def) and food restriction (Food-Def)) separated by 1 week. Thirty minutes of cycling at 64.5(3.2)% of maximum oxygen uptake was performed in Ex-Def from 0-0.5h, which induced an energy deficit of 1469(256)kJ. An equivalent energy deficit was induced in Food-Def (1478(275)kJ) by reducing the energy content of standardised test meals at 1h and 4h. Appetite ratings, acylated ghrelin and peptide $YY_{3-36}$ concentrations were measured throughout each trial. An *ad libitum* meal was provided at 7h. Appetite was higher in Food-Def than Ex-Def from 4-8h (P=0.033) and tended to be higher across the entire 8h trial (P=0.059). However, energy intake at the *ad libitum* meal did not differ between trials (P = 0.634; Con 4376 (1634); Food-Def 4481 (1846); Ex-Def 4217 (1850) kJ). Acylated ghrelin was not related to changes in appetite but plasma $PYY_{3-36}$ concentrations were higher in Ex-Def than Food-Def (P<0.05) and negatively correlated with changes in appetite across the entire 8h trial (P=0.037). An energy deficit of ~1475kJ stimulated compensatory increases in appetite when induced via calorie restriction but not when achieved by an acute bout of exercise. Appetite responses were associated with changes in plasma $PYY_{3-36}$ but not acylated ghrelin concentrations and did not influence subsequent energy intake.

**Keywords:** gastrointestinal hormones; acylated ghrelin; peptide YY; energy balance; compensation; energy intake

## Introduction

Obesity is characterised by an excess accumulation of body fat and is associated with an increased prevalence of chronic diseases including type 2 diabetes, osteoarthritis, cardiovascular disease and some forms of cancer (Bray, 2004). Consequently, overweight and obesity has recently been classified as one of the top five global risk factors for mortality and one of the top ten risk factors for morbidity (World Health Organisation, 2009). However, weight loss as little as 3 % has been associated with favourable changes in chronic disease risk factors and therefore represents a major public health priority (Donnelly et al., 2009).

For weight loss to occur, a sustained negative energy balance is required and is typically achieved by decreasing energy intake (i.e. dieting) and/or increasing energy expenditure (i.e. exercising). Although both interventions induce a negative energy balance, current research suggests that exercise and caloric restriction elicit contrasting homeostatic responses. In this regard, acute caloric restriction appears to stimulate rapid compensatory increases in appetite and energy intake that do not occur in response to equivalent energy deficits induced by exercise (Hubert et al., 1998; King et al., 2011a). Furthermore, King et al. (2011a) reported immediate decreases in circulating concentrations of the anorectic gut hormone $PYY_{3-36}$ and increases in the orexigenic gut hormone acylated ghrelin in response to food restriction but no compensatory changes in response to exercise. Such findings suggest that these appetite-regulating gut hormones have a mediating role in the immediate appetite and energy intake responses to acute energy deficits but this requires further investigation.

Although these studies have provided interesting information regarding energy homeostasis and the regulation of appetite, large and abrupt methods of energy restriction have been employed as calorie intake was reduced by ~1820 kJ at a single meal (Hubert et al., 1998) and ~4820 kJ across two meals (King et al., 2011a). Such substantial decreases in energy intake at individual meals increases the likelihood that compensatory increases in appetite will occur and does not represent a practical

strategy for energy restriction. In this regard, research has demonstrated that compensatory changes in gastrointestinal hormones and increases in appetite persist for at least one year after weight loss induced by a very low energy diet, despite increases in body weight (Sumithran et al., 2011).

The current UK government and American College of Sports Medicine (ACSM) guidelines recommend a minimum of 150 $min.wk^{-1}$ of moderate intensity physical activity, spread over most days of the week (British Heart Foundation, 2009; Donnelly et al., 2009). This may be interpreted as five 30 min exercise bouts performed on separate days of the week and is considered to be sufficient to reduce chronic disease risk, prevent significant weight gain, and elicit modest weight loss in overweight and obese populations (Donnelly et al., 2009). The appetite and energy intake response to such a practical energy deficit achieved via exercise and food restriction is unknown. This requires further investigation as compensatory increases in appetite contribute to the difficulty of maintaining an energy deficit in current society where energy dense, highly palatable foods are abundant and easily accessible. Furthermore, increases in appetite are commonly cited as a reason for unsuccessful dieting (Ikeda et al., 2004) and are inversely related to exercise-induced weight loss (King et al., 2008).

The purpose of this study was to investigate the appetite, acylated ghrelin, $PYY_{3-36}$ and energy intake responses to a 30 min bout of moderate intensity cycling compared with an equivalent energy deficit achieved via caloric restriction. This study also enables further investigation into the sensitivity of the appetite-regulating system and the role of acylated ghrelin and $PYY_{3-36}$ in energy homeostasis via the utilisation of small, yet practical, energy deficits. It was hypothesised that appetite and acylated ghrelin would increase, and that $PYY_{3-36}$ would decrease in response to food restriction but that these variables would remain unaffected by exercise, resulting in a higher energy intake in the food restriction trial.

**Methods**

*Participants*

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Loughborough University Ethics Advisory Committee (reference number: R12-P61). Written informed consent was obtained from all participants. Participants were male, non-smokers, not taking medication, weight stable for at least 6 months before the study and were not dieting. The physical characteristics of participants (mean (SD)) were as follows: age 24 (5) years, body mass index (BMI) 23.8 (2.7) $kg.m^{-2}$, body mass 75.3 (10.3) kg, body fat 14.2 (4.0) %, waist circumference 80.3 (6.6) cm, maximum oxygen uptake ($VO_2$ max) 55.4 (9.1) $mL.kg^{-1}.min^{-1}$.

*Preliminary Trials*

Prior to main trials participants visited the laboratory for two preliminary trials. During the first visit, preliminary anthropometric measurements were collected and participants completed a maximal exercise test to determine $VO_2$ max. Height and body weight were measured and BMI was subsequently calculated. Body fat percentage was estimated via skinfold measurements of the biceps, triceps, sub-scapular and suprailiac sites (Durnin & Womersley, 1974) and waist circumference determined as the narrowest part of the torso between the xiphoid process and the iliac crest. Maximum oxygen uptake was determined using a continuous incremental cycle test to exhaustion as described previously (Deighton et al., 2013a). Acceptability of the food items to be provided during the main trials was assessed by completion of a food preference questionnaire. The questionnaire required participants to rate preselected food items on a scale ranging from 1 (dislike extremely) to 10 (like extremely). Any volunteers that scored $\leq 5$ for any of the pre-selected food items to be presented were excluded from participating in the study.

Participants visited the laboratory on a second occasion for a familiarisation trial. Participants performed 30 min of continuous cycling exercise on an electromagnetically braked cycle ergometer

(Lode Excalibur Sport V2, Groningen, Netherlands) at a work rate predicted to elicit 65 % of $VO_2$ max. Samples of expired air were collected at 6, 18 and 30 min during exercise to monitor the intensity of the cycle bout, with adjustments made to the work rate if necessary. Heart rate (Polar T31; Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE) (Borg, 1973) were also measured at these times. Energy expenditure of exercise was calculated using the equation of Frayn (Frayn, 1983), for the determination of energy provision during the main trials.

*Experimental Protocol*

Participants performed three 8 h experimental trials (control, exercise-induced energy deficit and diet-induced energy deficit) separated by one week in a counterbalanced Latin Square design. Participants completed a weighed food diary in the 24 h before the first main trial and replicated this before each subsequent trial. Alcohol, caffeine and strenuous physical activity were not permitted during this period. Participants arrived at the laboratory at 0800 h after an overnight fast of at least 10 h and exerted themselves minimally when travelling to the laboratory, using motorized transport when possible. Verbal confirmation of dietary and exercise standardisation was obtained at the beginning of each experimental trial.

During each trial, appetite perceptions (hunger, satisfaction, fullness and prospective food consumption) (Flint et al., 2000) were assessed at baseline, 0.25, 0.5 h and every 30 min thereafter using 100 mm visual analogue scales. An overall appetite rating was calculated as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al., 2000).

*Test Meals*

At 1 h (~9am) participants were provided with a standardised breakfast, which consisted of toasted white wheatgerm bread, margarine, strawberry jam, banana and orange juice. The macronutrient

content of the meal was 72.9 % carbohydrate, 9.5 % protein and 17.6 % fat. A standardised lunch was provided at 4 h (~12pm) and consisted of a tuna and mayonnaise sandwich, salted crisps, chocolate muffin and green apple. The macronutrient content of the meal was 47% carbohydrate, 17.6% protein and 35.4% fat.

*Energy Deficits*

Participants rested within the laboratory throughout all trials (sitting reading, working at a desk or watching television), except from 0 – 0.5 h during the exercise-induced energy deficit (Ex-Def) trial where participants replicated the exercise bout performed during the familiarisation trial. To calculate the net energy expenditure of exercise (gross energy expenditure of exercise minus energy expenditure at rest), expired gas was collected into Douglas bags for 5 min every 10 min between 0 and 0.5 h during the control (Con) and diet-induced energy deficit (Food-Def) trials (Frayn, 1983).

The energy content of the test meals was identical in Con and Ex-Def. The breakfast meal provided 30 % and the lunch meal 35 % of the estimated daily energy needs of each individual for a sedentary day, which was calculated using the Mifflin-St Jeor equation and a physical activity factor of 1.4 (Mifflin et al., 1990). The mean (SD) energy intake at breakfast and lunch in Con and Ex-Def was 3074 (221) kJ and 3587 (258) kJ. This equated to a breakfast composition of: 171.3 (12.3) g bread, 17.1 (1.2) g margarine, 40.0 (2.9) g strawberry jam, 114.2 (8.2) g banana and 171.3 (12.3) g orange juice. The average lunch composition was as follows: 103.4 (7.4) g bread, 11.4 (0.8) g mayonnaise, 96.8 (7.0) g tuna, 17.9 (1.3) g salted crisps, 70.5 (5.1) g chocolate muffin and 121.3 (8.7) g apple.

In Food-Def, the energy content of the test meals was reduced by deducting the net energy expenditure of exercise from the energy provided at the test meals during Con and Ex-Def. This energy deficit was individually prescribed based on energy expenditure data and the total amount of

energy deducted was divided proportionally between the breakfast and lunch meals. Therefore, equivalent energy deficits were induced in Ex-Def and Food-Def relative to Con.

*Ad Libitum Meal*

At 7 h (~3pm) an *ad libitum* meal was provided, consisting of fusilli pasta that was cooked in a microwave for 12 min in unsalted water and served in a bolognaise sauce. For all meals, 600 g of dry pasta was prepared with 333 g of bolognaise sauce. The macronutrient composition of the meal was 77.5% carbohydrate, 13.8% protein and 8.7% fat. The energy density of the meal was 5.8 (0.4) $kJ.g^{-1}$. Participants were provided with a small bowl, which was repeatedly filled with the pasta meal before the participant had emptied it in an attempt to blind the participant to the amount of food eaten. No time limit was set for eating and participants were instructed to eat until 'comfortably full'. Each participant consumed the meal separately in the presence of a sole experimenter and any discussions about food were avoided. Food intake was determined as the weighted difference in food before and after eating and energy intake was subsequently determined using manufacturers' values. Water was available *ad libitum* and recorded throughout each trial.

*Blood Sampling*

Upon arrival to the laboratory, participants rested in a semi-supine position and a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Blood samples were collected at baseline, 1, 2.5, 4, 5, 6, 7 and 8 h for the determination of plasma acylated ghrelin and $PYY_{3-36}$ concentrations. To prevent the degradation of acylated ghrelin, blood samples were collected into pre-chilled 4.9 mL monovettes containing a 50 µl solution of potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH). These monovettes were spun at 1165 x *g* for 10 min at 4°C. The plasma supernatant was then dispensed into a storage tube and 100 µl of 1M hydrochloric acid was added per millilitre of plasma to

preserve acylated ghrelin (Hosoda et al., 2004). Thereafter, samples were spun at 1165 x *g* for 5 min at 4°C prior to storage at -20°C.

For the determination of plasma $PYY_{3-36}$ concentrations, blood samples were collected into pre-chilled syringes containing 10 µl DPP-IV inhibitor (Millipore, Watford, UK) per mL of blood. Syringes were then inverted and the blood dispensed into pre-chilled 2 mL EDTA tubes containing 500 KIU aprotonin (Nordic Pharma, Reading, UK) per mL of blood. Blood tubes were promptly centrifuged at 1165 × *g* for 10 min at 4 °C. The plasma supernatant was stored at -20°C for later analysis.

All samples were collected in the semi-supine position. Measurements of haemoglobin and haematocrit were taken to estimate changes in plasma volume (Dill & Costill, 1974). The mean coefficient of variation for blood haemoglobin and haematocrit measures was 0.9 % and 0.8 %, respectively.

*Biochemical Analysis*

A commercially available enzyme immunoassay was used to determine plasma concentrations of acylated ghrelin (SPI BIO, Montigny le Bretonneux, France). Plasma concentrations of $PYY_{3-36}$ were determined using a commercially available radioimmunoassay (Millipore, Watford, UK). To eliminate interassay variation, samples from each participant were analysed in the same run. The within batch coefficient of variation for the assays were 6.8 and 7.2 % for acylated ghrelin and $PYY_{3-36}$, respectively.

*Statistical Analysis*

Data was analysed using IBM SPSS statistics version 19 for Windows. Area under the curve (AUC) values were calculated using the trapezoidal method. One-way repeated measures ANOVA was used to assess trial-based differences in energy intake at the ad libitum meal as well as baseline and

AUC values for appetite, acylated ghrelin and $PYY_{3-36}$. Where significant main effects of trial were found, post-hoc analysis was performed using Holm-Bonferroni correction for multiple comparisons. In accordance with previous research (Deighton et al., 2013b; Stoeckel et al., 2008) acylated ghrelin and $PYY_{3-36}$ concentrations are presented as delta values in order to minimise the influence of day-to-day biological variations in these hormones. Correction of acylated ghrelin and $PYY_{3-36}$ concentrations for changes in plasma volume did not alter the interpretation of the results; therefore, for simplicity, the unadjusted values are presented. Statistical significance for this study was accepted as $P < 0.05$. Results in text and tables are presented as mean (SD). Graphical representations of results are presented as mean (SEM) to avoid distortion of the graphs. Based on previous data from our laboratory (Deighton et al., 2013a), a sample size of 12 participants was determined as sufficient to detect a 10 % difference in appetite perceptions during the post-exercise period. This calculation was performed using G*power with an alpha value of 5 % and a power of 80 % (Faul et al., 2007).

## Results

### *Exercise responses*

Participants completed the 30 min cycle at 186 (38) W. This elicited an oxygen consumption equivalent to 64.5 (3.2) % of $VO_2$ max and a net energy expenditure of 1469 (256) kJ. The non-protein respiratory exchange ratio was 0.93 (0.04), which reflected a proportional contribution to energy provision of 78 (13) % carbohydrate and 22 (13) % fat. Heart rate and RPE were 156 (16) $beats.min^{-1}$ and 13 (1), respectively.

### *Appetite*

Overall appetite ratings did not differ between trials at baseline (Con 74 (14); Food-Def 74 (14); Ex-Def 77 (10); $P = 0.735$). One-way ANOVA revealed a main effect of trial for appetite AUC

from 4 – 8 h (P = 0.021). Subsequent post-hoc analysis demonstrated significantly higher appetite in Food-Def than Ex-Def (P = 0.033). Appetite AUC did not differ between trials for 0 – 1 h and 1 – 4 h but tended to be higher in Food-Def than Ex-Def across the entire 8 h trial (P = 0.059; Figure 1; Table 1).

*Energy intake*

The combined energy intake of the breakfast and lunch test meals was 6661 (479) kJ in Con and Ex-Def and 5183 (378) kJ in Food-Def. Consequently, the energy deficit induced by food restriction was 1478 (275) kJ. This was comparable with the energy deficit induced through exercise (1469 (256) kJ; Paired samples t-test, P = 0.60).

One-way ANOVA revealed no between trial differences in the amount of food consumed at the *ad libitum* meal (P = 0.760; Con 764.6 (295.4); Food-Def 765.9 (307.7); Ex-Def 734.5 (313.4) g). Consequently energy intake did not differ between trials (P = 0.634; Con 4376 (1634); Food-Def 4481 (1846); Ex-Def 4217 (1850) kJ). This resulted in an energy balance that was 1628 (915) kJ and 1373 (1047) kJ lower in Ex-Def and Food-Def compared with Con (both $P \leq 0.001$).

There was a significant main effect of trial for *ad libitum* water intake (P = 0.049). Post-hoc analysis demonstrated a tendency for greater water consumption across the Ex-Def trial compared with Con and Food-Def (Con 901 (445); Food-Def 710 (422); Ex-Def 1181 (679) mL).

*Plasma acylated ghrelin concentrations*

Fasting plasma acylated ghrelin concentrations did not differ significantly between trials at baseline (Con 189 (262); Ex-Def 242 (386); Food-Def 268 (427) $pg.mL^{-1}$; P = 0.174). Delta AUC for acylated ghrelin concentrations tended to be higher in Con than Ex-Def and Food-Def from 0-1 h (P = 0.081) but did not differ between trials for any other time period (1-4 h: P = 0.116; 4-8 h: P = 0.217; 0-8 h: P = 0.160; Figure 2a).

Subsequent boxplot analysis of acylated ghrelin AUC values revealed three consistently outlying participants within the data set (Field, 2009). These participants exhibited fasting acylated ghrelin concentrations that were between 6 and 39 standard deviations higher than the mean fasting value of the remaining nine participants on all trials. In accordance with previous research, these three participants were removed from the data set for subsequent analysis (Broom et al., 2007; Hansen et al., 2002; King et al., 2011b). After the removal of these participants from the data, one-way ANOVA revealed significantly lower delta acylated ghrelin concentrations from 0 – 1 h in Ex-Def compared with Con and Food-Def ($P < 0.05$). There was also a tendency for depressed values in Ex-Def compared with Con and Food-Def from 1 – 4 h ($P = 0.069$) and across the entire 8 h trial ($P = 0.075$) (Figure 2b). Removal of the outliers did not affect the interpretation of the appetite or $PYY_{3-36}$ findings. Plasma acylated ghrelin concentrations for one outlying participant are displayed in Figure 2c in order to highlight the variation in acylated ghrelin profiles.

*Peptide $YY_{3-36}$ concentrations*

Fasting $PYY_{3-36}$ concentrations did not differ significantly between trials at baseline (Con 93.5 (40.0); Ex-Def 87.1 (37.9); Food-Def 96.7 (46.0) $pg.mL^{-1}$; $P = 0.325$). Delta AUC for plasma $PYY_{3-36}$ concentrations were significantly higher in Ex-Def than Con and Food-Def from 0 – 1 h ($P < 0.01$) and in Ex-Def compared with Food-Def from 1 - 4 h and across the entire 8 h trial ($P < 0.05$) (Figure 3; Table 2).

*Correlations*

Area under the curve values for delta $PYY_{3-36}$ concentrations were negatively correlated with changes in appetite for 0 - 1 h ($r = -0.514$; $P = 0.001$), 4 - 8 h ($r = -0.340$; $P = 0.043$) and for the entire 8 h trial (0 - 8 h; $r = -0.349$; $P = 0.037$). There were no significant correlations between acylated ghrelin and appetite AUCs for any time period. The *ad libitum* energy intake response to exercise and food restriction was not significantly correlated with any of the participant

characteristics including age, height, weight, BMI, body fat, waist circumference and $VO_2$ max (all $P > 0.18$).

## Discussion

The primary finding of this investigation is that an energy deficit of ~1475 kJ stimulated compensatory increases in appetite when induced via food restriction but not when achieved by an acute bout of exercise. These divergent appetite responses were associated with changes in circulating concentrations of $PYY_{3-36}$ but were unrelated to changes in plasma acylated ghrelin and did not influence subsequent energy intake.

This study has extended the findings of previous research by demonstrating that appetite perceptions increase in response to subtle reductions in energy intake but do not change in response to an equivalent exercise-induced energy deficit (Hubert et al., 1998; King et al., 2011a). Increases in appetite occurred despite an average decrease in energy intake of only 682 kJ at breakfast and 796 kJ at lunch. This highlights the sensitivity of the appetite-regulating system to reductions in food intake and supports previous observations that dieting is often compromised by increases in appetite (Ikeda et al., 2004). Additionally, the observed increase in appetite in response to food restriction across two meals was smaller than that previously reported for a similar energy deficit induced at a single meal (Hubert et al., 1998). This suggests that creating an energy deficit across multiple meals may be more effective for minimising increases in appetite than at a single meal but this requires further investigation. In contrast, appetite was unaltered in response to an equivalent energy deficit induced through 30 min of moderate intensity exercise. This exercise bout represents the current UK government and ACSM guidelines for physical activity (British Heart Foundation, 2009; Donnelly et al., 2009) and supports previous findings that an acute bout of continuous moderate intensity exercise does not stimulate compensatory increases in appetite during the subsequent hours (Deighton & Stensel, 2014).

In contrast with previous findings, the divergent appetite response to exercise and food restriction was not associated with concordant changes in plasma acylated ghrelin concentrations (King et al., 2011a). Furthermore, the acylated ghrelin profile of the participant displayed in Figure 2c exhibited an increase in response to the lunch meal in all trials despite reporting a simultaneous decrease in appetite. Such disassociation between appetite and ghrelin profiles in a single participant has previously been reported by Cummings et al. (2004), as one out of six participants did not demonstrate an increase in ghrelin prior to spontaneous meal request, despite exhibiting significant increases in appetite and a similar energy intake and meal request response as all other participants. The reasons for the occurrence of outlying participants in the present study are unclear as all outliers displayed an appetite, energy intake and $PYY_{3-36}$ response that was consistent with the remainder of the sample. Furthermore, there was no difference between the outlying and non-outlying participants for any of the measured physiological characteristics. In order to further investigate the mechanisms underlying the disassociation between appetite perceptions and ghrelin concentrations in some participants, it may be beneficial for future experiments to also measure circulating insulin levels as an inverse relationship between ghrelin and insulin concentrations has been previously reported (Cummings et al., 2004; Flanagan et al., 2003).

The removal of outlying participants from the acylated ghrelin data revealed a marked suppression of this peptide during the hours after exercise, which supports the findings of previous authors (Broom et al., 2007; Kawano et al., 2013; Wasse et al., 2013). However, contrary to the hypothesis of the study and previous findings from our laboratory (King et al., 2011a), food restriction did not stimulate any compensatory increases in acylated ghrelin. This is likely to reflect the smaller food restriction employed in the present study as a similar reduction in energy intake of ~1218 kJ did not influence 24 h total ghrelin concentrations in a previous investigation (Weigle et al., 2003).

The findings of the present study contribute to the current debate about the importance of physiological changes in ghrelin as a mediator of appetite. In this regard, a recent study by Lippl

328 and colleagues (2012) reported that exogenous infusion of ghrelin at physiological and mildly
329 supraphysiological doses does not influence appetite, spontaneous meal request or energy intake.
330 Furthermore, recent studies of knockout mice that are deficient for either ghrelin, the growth
331 hormone secretagogue receptor (GHS-R) or ghrelin-O-acyltransferase reported a similar feeding
332 response between these knockout mice and wild type controls (Sun et al., 2008; Zhao et al., 2010).
333 Alternatively, these authors suggested that the primary function of acylated ghrelin was to preserve
334 blood glucose concentrations during food restriction as an absence of either acylated ghrelin or
335 GHS-R elicited a significant reduction in blood glucose during 50 – 60 % calorie restriction. It
336 seems plausible that the 69 % calorie restriction employed by King et al. (2011a) may have
337 stimulated increases in acylated ghrelin to maintain blood glucose concentrations, whereas the 22 %
338 energy deficit in the present study may have been insufficient to threaten blood glucose levels.
339 Although this contributes to an interesting debate about the primary function of acylated ghrelin,
340 these suggestions are speculative and require further investigation.

341 Alternatively, changes in $PYY_{3-36}$ concentrations were significantly negatively correlated with
342 changes in appetite from 0 - 1 h, 4 - 8 h and for the entire 8 h trial. To the authors' knowledge, only
343 three experiments have previously measured the $PYY_{3-36}$ response to exercise beyond the provision
344 of a single test meal (Cheng et al., 2009; Deighton et al., 2013b; King et al., 2011a). The findings of
345 the present study support previous findings by demonstrating a prolonged increase in $PYY_{3-36}$ after
346 exercise. Furthermore, although not statistically significant, the increase in $PYY_{3-36}$ concentrations
347 in response to the lunch meal appeared to be reduced during the food restriction trial. Considering
348 the prominent role of $PYY_{3-36}$ as a mediator of satiety (Batterham et al., 2007), it seems plausible
349 that the contrasting changes in $PYY_{3-36}$ in response to exercise and food restriction may be
350 implicated in the divergent appetite response to these trials. However, it must be noted that appetite
351 is regulated by the complex interaction of many physiological and psychological factors (King et
352 al., 2007; Murphy & Bloom, 2006). Therefore, the response of a single hormone to the subtle
353 energy deficits employed in this study is unlikely to account for all of the variation in appetite

between trials. Nevertheless, considering that obese participants have consistently been found to exhibit a blunted PYY and satiety response to feeding (Batterham et al., 2006; Korner et al., 2005; Stock et al., 2005; le Roux et al., 2006), it would be useful for future experiments to investigate whether this response is improved with exercise.

Surprisingly, despite a significant increase in appetite in response to caloric restriction, energy intake at the *ad libitum* meal did not differ between trials. This contrasts with previous investigations that have demonstrated an increase in energy intake in response to food restriction compared with an equivalent energy deficit induced via exercise (Hubert et al., 1998; King et al., 2011a). However, this is likely to reflect the smaller changes in appetite observed in the present study due to the modest energy deficits employed. Such a disassociation between appetite and energy intake has been commonly reported within the scientific literature in response to modest experimental manipulations and is thought to represent an accruing degree of motivation prior to the initiation of a behavioural response (Stubbs et al., 2000). The uncoupling between appetite perceptions and energy intake may also have been influenced by the sedentary activities of participants between the lunch and *ad libitum* meal. In this regard, research has demonstrated that sedentary activities can stimulate hedonic feeding (Chaput et al., 2011), which is likely to have occurred in the present study considering the large *ad libitum* energy intakes despite appetite scores immediately prior to the meal being rated as ~ 60 out of 100. Such high energy intakes may have reduced the sensitivity of the meal to detect changes in energy intakes as a result of the exercise and caloric restriction interventions. It seems reasonable to speculate that continued food restriction would elicit increases in energy intake over a longer monitoring period but this requires further investigation.

Although closely supervised interventions involving either exercise alone or dieting alone have been demonstrated to result in successful weight loss (King et al., 2008; Stewart & Fleming, 1973), these interventions are largely unsuccessful when the participants are not closely supervised (Franz

et al., 2007). This is likely to reflect a lack of adherence as changes in exercise participation and dietary practises represent challenging interventions for many individuals. In this regard, the findings of the present study have demonstrated the sensitivity of the appetite-regulating system to reductions in food intake, which emphasises the need for significant willpower to resist increases in appetite during food restriction. Alternatively, fulfilment of the current physical activity guidelines requires a significant lifestyle change, time commitment and level of exertion for a sedentary individual. In this regard, 30 min of exercise that was perceived as 'somewhat hard' only induced an energy deficit of ~1469 kJ in the present study, which highlights the substantial time commitment that is required to induce larger energy deficits using exercise alone. Furthermore, due to the high fitness levels of participants in the present study, the energy expenditure achieved during exercise is likely to be in excess of that achieved by sedentary participants exercising at the same relative intensity. Considering that the energy deficits utilised in the present study are below the recommended minimum of 2092 $kJ.d^{-1}$ for weight loss (NHS Choices, 2011) and that larger energy deficits are required for greater weight loss, it seems logical to encourage a combined exercise and dietary approach to weight loss in order to compromise between the difficulties of each individual intervention. This supports findings from systematic reviews that combined diet and exercise interventions are the most effective non-surgical method of achieving sustained weight loss (Curioni & Lourenço, 2005; Franz et al., 2007). Furthermore, in addition to creating a more tolerable energy deficit, the inclusion of exercise to complement an energy-restricted diet has been found to preserve muscle mass during weight loss. This is particularly important for addressing the growing health concern of 'sarcobesity', which is characterised by a concomitant increase in fat mass and decrease in muscle mass (Parr et al., 2013).

Although the findings of the present study have contributed to our understanding of the appetite response to exercise and food restriction, this study also contains some notable limitations. Firstly, the population sample was limited to a small number of healthy active men; therefore the findings may not generalise to other populations. Although previous research suggests that exercise elicits

similar appetite and energy intake responses in lean and obese participants (Ueda et al., 2009), further investigations in overweight and obese populations are needed because this is where weight-management strategies have the most clinical relevance. Additionally, due to the time-constraints of the present study, ad libitum feeding occurred ~3 h after the standardised lunch meal when appetite remained relatively low. This does not represent an ecologically valid scenario and increases the likelihood that food intake during this meal was driven by hedonic rather than homeostatic stimuli. Additionally, the use of a single food item to assess *ad libitum* energy intake prevented any investigation into the effects of the interventions on food choice. However, the use of a single food item allowed a more consistent evaluation of energy intake as the macronutrient content of the meal was fixed. Finally, the mechanistic investigation of this study was limited to the measurement of acylated ghrelin and $PYY_{3-36}$. Future studies may aim to assess changes in additional gastrointestinal hormones including glucagon-like-peptide-1 (GLP-1), pancreatic polypeptide and oxyntomodulin. The measurement of GLP-1 in combination with $PYY_{3-36}$ may be particularly prudent as these hormones have been found to have an additive effect on satiety (De Silva et al., 2011).

In conclusion, food restriction of ~1478 kJ across two meals stimulated compensatory increases in appetite that did not occur in response to a similar energy deficit induced by 30 min of moderate intensity exercise. Although the mechanisms underlying such a contrasting response are unclear, it does not appear to be influenced by changes in plasma acylated ghrelin concentrations. Alternatively, changes in $PYY_{3-36}$ were negatively correlated with changes in appetite, which supports the anorexigenic nature of this peptide. Future studies should be conducted to elucidate whether $PYY_{3-36}$ concentrations also increase in response to exercise in obese participants and if this improves the satiety response to a standardised meal.

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Table 1. Time-averaged area under the curve values for overall appetite perceptions in the Control, Ex-Def and Food-Def trials.

| | **Preprandial (0 - 1 h)** | **Morning ( 1- 4 h)** | **Afternoon (4 - 8 h)** | **Total trial (0 - 8 h)** |
|---|---|---|---|---|
| **Overall Appetite (0 - 100)** | | | | |
| Control | 76 (14) | 49 (16) | 40 (13) | 48 (13) |
| Ex-Def | 70 (14) | 53 (13) | 39 (11) | 48 (11) |
| Food-Def | 78 (12) | 57 (15) | 46 (14) | 54 (13) |
| P | 0.386 | 0.120 | 0.021* | 0.059 |

Values are mean (SD), N = 12. *Different between Ex-Def and Food-Def (One-way ANOVA: $P < 0.05$ after Holm-Bonferroni adjustment).

Table 2. Time-averaged area under the curve values for delta $PYY_{3-36}$ concentrations in the Control, Ex-Def, and Food-Def trials.

| | **Preprandial (0 – 1 h)** | **Intertest meal (1 – 4 h)** | **Posttest meals (4 – 8 h)** | **Total Trial (0 – 8 h)** |
|---|---|---|---|---|
| **Delta $PYY_{3-36}$ ($pg.mL^{-1}$)** | | | | |
| Control | -4.1 (8.3) | 3.1 (21.0) | 21.4 (34.7) | 11.3 (25.1) |
| Ex-Def | 7.3 (5.7) | 19.7 (16.9) | 35.4 (24.2) | 26.0 (17.7) |
| Food-Def | -5.9 (5.8) | 2.9 (11.1) | 14.6 (21.2) | 7.7 (12.8) |
| P | $< 0.0005^{*\dagger}$ | $0.039^{*}$ | 0.086 | 0.036* |

Values are mean (SD), N = 12. *Different between Ex-Def and Food-Def, †Different between Ex-Def and Control (One-way ANOVA: $P < 0.05$ after Holm-Bonferroni adjustment).

Figure 1. Overall appetite perceptions in Con (▼), Ex-Def (●) and Food-Def (○). Values are mean (SEM), N = 12. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

Figure 2. Delta plasma acylated ghrelin concentrations in Con (▼), Ex-Def (●) and Food-Def (○) presented for all participants (a), after the removal of three outlying participants (b) and presenting the values of a single outlying participant (c). Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

Figure 3. Delta $PYY_{3-36}$ concentrations in Con (▼), Ex-Def (●) and Food-Def (○). Values are mean (SEM), N = 12. Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates ad libitum meal.

**Figure 1**

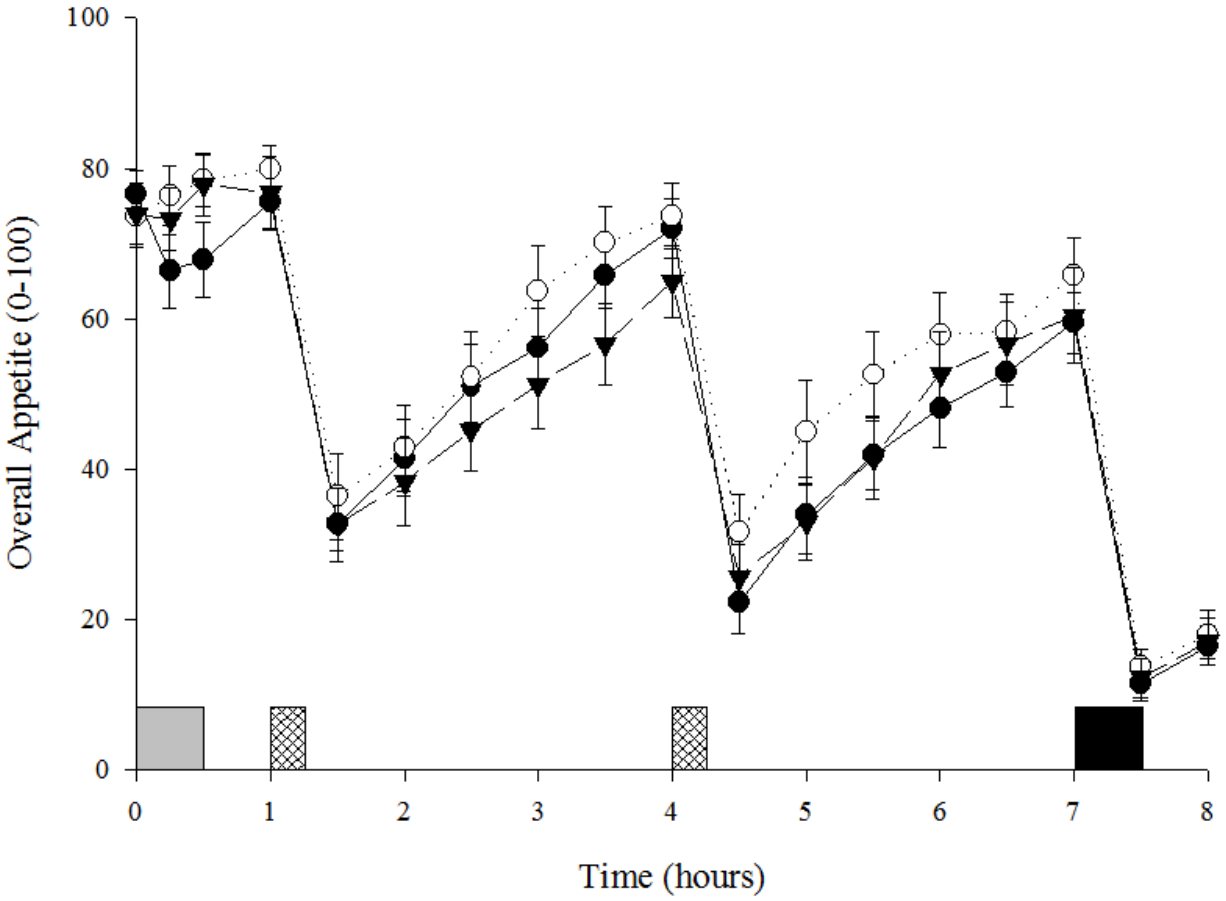


**Figure 2**

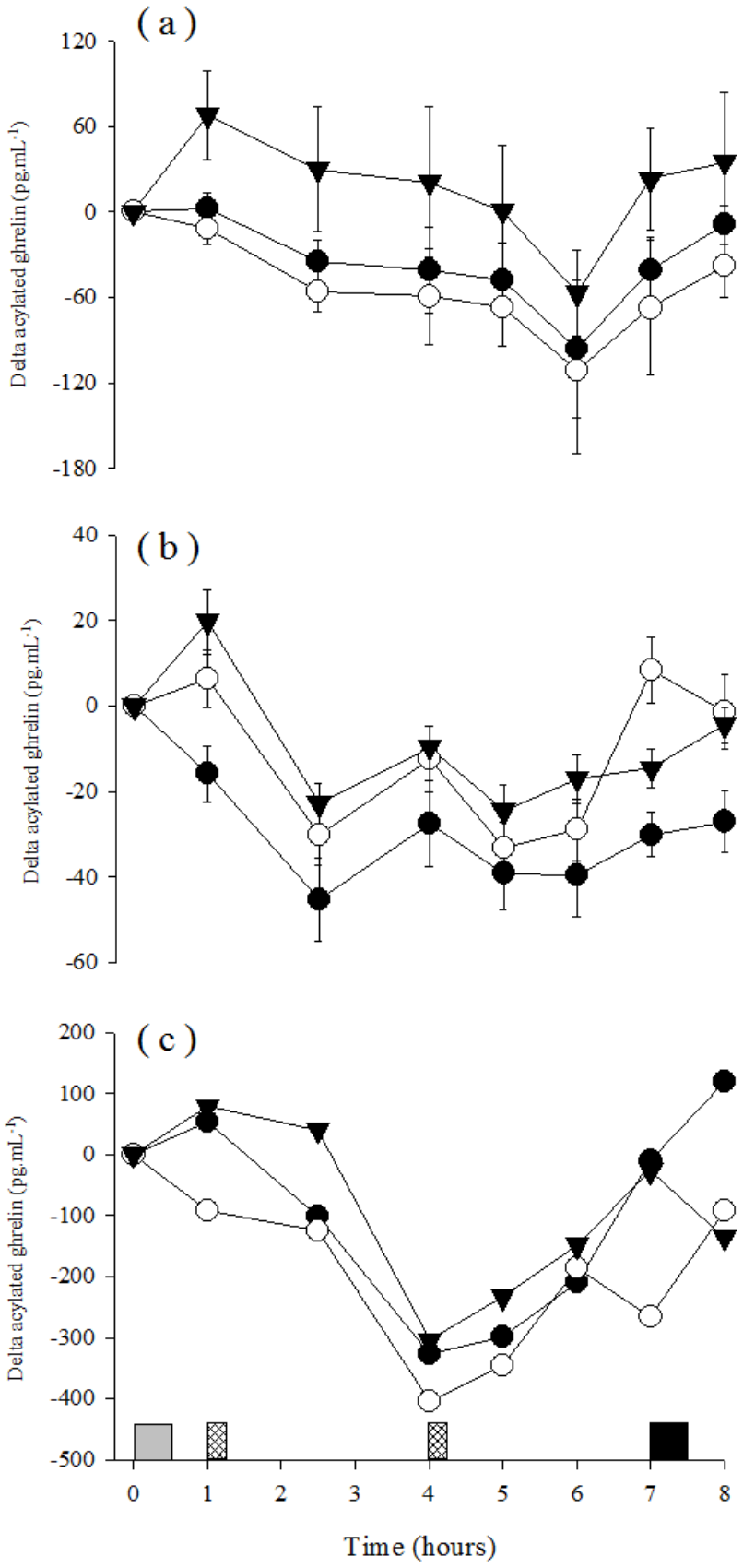
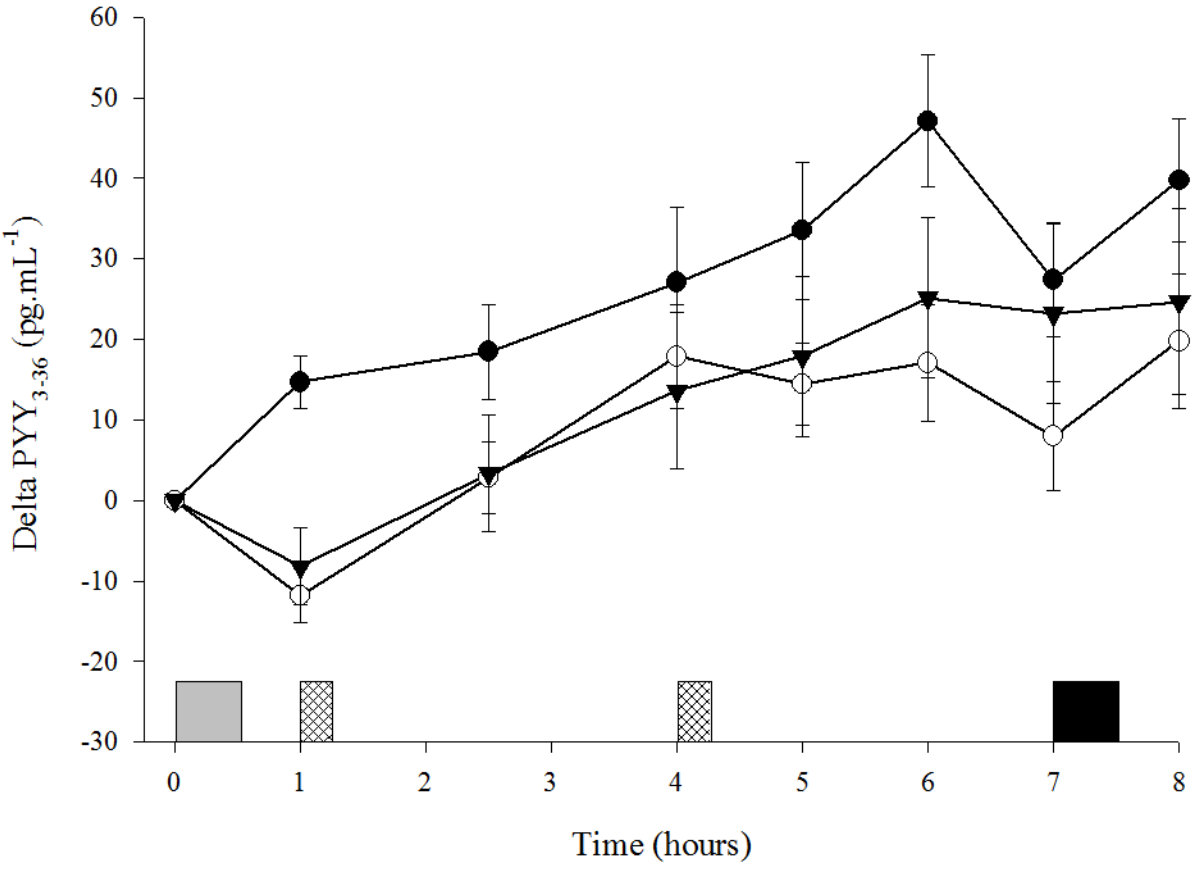

( a )
120
60
0
-60
-120
-180
Delta acylated ghrelin (pg.mL-1)
( b )
40
20
0
-20
-40
-60
Delta acylated ghrelin (pg.mL-1)
( c )
200
100
0
-100
-200
-300
-400
-500
Delta acylated ghrelin (pg.mL-1)
0
1
2
3
4
5
6
7
8
Time (hours)

**Figure 3**


60
50
40
30
20
10
0
-10
-20
-30
Delta PYY3-36 (pg.mL-1)
0
1
2
3
4
5
6
7
8
Time (hours)