APPETITE AND ENERGY INTAKE RESPONSES TO ACUTE ENERGY DEFICITS IN FEMALES VERSUS MALES

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ABSTRACT

Purpose: To explore whether compensatory responses to acute energy deficits induced by exercise or diet differ by sex. **Methods:** In experiment one, twelve healthy women completed three 9 h trials (control, exercise-induced (Ex-Def) and food restriction induced energy deficit (Food-Def)) with identical energy deficits being imposed in the Ex-Def (90 min run, ~70% of VO_2 max) and Food-Def trials. In experiment two, 10 men and 10 women completed two 7 h trials (control and exercise). Sixty min of running (~70% of VO_2 max) was performed at the beginning of the exercise trial. Participants rested throughout the remainder of the exercise trial and during the control trial. Appetite ratings, plasma concentrations of gut hormones and *ad libitum* energy intake were assessed during main trials. **Results:** In experiment one, an energy deficit of ~3500 kJ induced via food restriction increased appetite and food intake. These changes corresponded with heightened concentrations of plasma acylated ghrelin and lower peptide $YY_{3\text{-}36}$. None of these compensatory responses were apparent when an equivalent energy deficit was induced by exercise. In experiment two, appetite ratings and plasma acylated ghrelin concentrations were lower in exercise than control but energy intake did not differ between trials. The appetite, acylated ghrelin and energy intake response to exercise did not differ between men and women. **Conclusions:** Women exhibit compensatory appetite, gut hormone and food intake responses to acute energy restriction but not in response to an acute bout of exercise. Additionally, men and women appear to exhibit similar acylated ghrelin and $PYY_{3\text{-}36}$ responses to exercise-induced energy deficits. These findings advance understanding regarding the interaction between exercise and energy homeostasis in women.

KEY WORDS: sex-based differences; gastrointestinal hormones; compensation; energy balance; females

INTRODUCTION

The regulation of appetite control and energy balance is an area of scientific enquiry which continues to receive widespread attention across disciplines. To date, as in many fields of science, the foundation of our knowledge within appetite regulation has been gleaned from studies conducted predominantly in men. Consequently, less in known specifically regarding the regulation of appetite control and energy balance in women and the potential for sex-based differences has not been thoroughly investigated. Preliminary research has hinted that appetite and appetite-regulatory hormones may display divergent responses to nutritional interventions between men and women however this proposition continues to be debated (5). Specifically, compared to men, it has been suggested that women exhibit more potent compensatory responses (appetite, appetite regulatory hormones, food intake) to energy deficits in order to preserve energy balance and reproductive function (14). This viewpoint is supported by studies demonstrating that men exhibit greater reductions in body fat and body mass than women in response to supervised exercise training (8,19,35). Conversely, other research has suggested that differences in weight loss and adiposity responses to exercise are unrelated to sex (5,6).

Sex-based differences in the short-term regulation of appetite and energy balance were previously investigated in a carefully designed experimental study using consecutive days of exercise to induce an energy deficit in male and female participants (15). The researchers showed that this acute exercise-induced energy deficit triggered a compensatory increase in circulating acylated ghrelin (appetite stimulating hormone) in women but not in men. These changes corresponded with higher appetite ratings in women than men and suggest that sex-based differences may be apparent in the early appetite and gut hormone response to exercise-induced energy deficits.

Over the past decade our laboratory has conducted many acute experimental trials seeking to enhance understanding concerning the short-term regulation of appetite and energy balance (21,31). In a sample of male participants, we recently demonstrated that the induction of an acute energy deficit by food restriction elicited a rapid and robust compensatory appetite, gut hormone (acylated ghrelin and PYY_{3-36}) and energy intake response whilst the same energy deficit imposed by exercise had no effect (20). These findings suggest that the method by which an energy deficit is imposed has a marked impact on the subsequent physiological and behavioural response. It is currently unknown whether women exhibit the same acute responses to exercise and food restriction as men. This information has important implications regarding the utility of lifestyle therapies to assist weight control in women.

Within this report we describe the findings from two acute experimental studies which sought to provide new information regarding the short-term appetite, food intake and appetite hormone responses to exercise and food-induced energy deficits in men and women. In experiment one; we compared the appetite, energy intake, acylated ghrelin and PYY_{3-36} responses to an equivalent energy deficit induced by exercise or energy restriction in women. In experiment two, we directly compared appetite, food intake and circulating acylated ghrelin responses to an exercise-induced energy deficit in men verses women. Our findings identify a high degree of similarity in the acute response to energy deficits in men and women.

METHODS

Experimental protocol

This investigation contained two experiments which were conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures were approved by the

Institutional Ethics Advisory Committee and written informed consent was obtained from all participants. Study participants were non-smokers, not taking medication, weight stable for at least six months before participation and were not dieting. Participants had no known history of cardiovascular/metabolic disease and with respect to female participants, were of reproductive age but were not pregnant. In each study, participants were recreationally active i.e. were familiar with exercise, but were not formally trained in endurance activities such as running or cycling.

Participants completed a weighed food diary in the 24 h before the first main trial of each experiment and replicated this before each subsequent trial. Alcohol, caffeine and strenuous physical activity were not permitted during this period. All trials commenced between 8am and 9am after an overnight fast of at least 10 h and participants exerted themselves minimally when travelling to the laboratory, using motorised transport when possible. Verbal confirmation of dietary and exercise standardisation was obtained at the beginning of each experimental trial. Female participants completed all main trials within the follicular phase of the menstrual cycle (4).

Preliminary trials

In order to determine the running speed required to elicit 70% of maximum oxygen uptake (VO_2 max) for each individual, participants completed a preliminary trial before the main trials for each experiment. This consisted of a submaximal running test and a VO_2 max test on a motorised treadmill (34). Anthropometric measurements and study questionnaires e.g. Three Factor Eating Questionnaire (TFEQ) (33) was also taken/completed at this time. At this visit, participants also verbally confirmed acceptability of the test meals and ad *libitum* meals subsequently to be provided during main experimental trials.

In experiment one a second preliminary trial was completed to determine the net energy cost of exercise which was needed to calculate food provision in the main trials and to enable trial randomisation in advance. During this session participants ran for 90 min at 70% of VO_2 max with expired air samples being collected into Douglas bags at 15 min intervals to calculate energy expenditure using the equations provided by Frayn (12).

Experiment One

Twelve female participants performed three 9 h experimental trials (control (Con)), exercise-induced energy deficit (Ex-Def) and diet-induced energy deficit (Food-Def)) separated by one-week in a randomised counterbalanced design. To ensure standardisation of menstrual phase, participants' first main trial was undertaken at the beginning of their follicular phase with their second trial occurring one-week later. Participants' third main trial was subsequently undertaken at the beginning of their next cycle approximately four weeks later. Participants rested within the laboratory throughout all trials with participants being permitted to read, work at a computer or watch DVDs which had been screened to ensure that there was no overt emphasis on food and drink. The exception to this occurred at 0-1.5 h during Ex-Def where participants performed 90 min of treadmill running at ~70% of VO_2 max (identical to that performed during the preliminary trial). Resting expired air samples were collected from 0 – 1.5 h during the Con and Food-Def trials to calculate the net energy expenditure of exercise (gross energy expenditure of exercise minus energy expenditure at rest) (12).

Identical test meals were provided at 2 h (breakfast) and 4.75 h (lunch) and were each consumed within 15 min. The meals consisted of a tuna and mayonnaise sandwich, salted crisps, chocolate muffin and green apple. The macronutrient composition of the meal was 47% carbohydrate, 18% protein and 35% fat. The energy content of the test meals was identical in Con and Ex-Def (2778 (109) kJ) with each meal providing 35% of participants' estimated daily energy needs for a sedentary day. This calculation was based upon an estimation of each participant's daily energy needs which was determined using a validated equation for resting metabolic rate (28) that was multiplied by an activity factor (1.4) deemed appropriate for a sedentary day (10). In Food-Def, the energy content of the test meals was reduced (1025 (159) kJ) 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 the exercise energy expenditure data derived from the preliminary trials and the total amount of energy deducted was divided equally between breakfast and lunch. Therefore, equivalent energy deficits were induced in Ex-Def and Food-Def relative to Con. The macronutrient percentage of the test meals was identical across main trials i.e. only the meal energy content was altered in the Food-Def trial.

Experiment Two

Ten female and 10 male participants performed two 7 h experimental trials (exercise and control) separated by one week in a randomised counterbalanced design. Female participants completed both main trials during the follicular phase (days 1 – 11) of their menstrual cycle. Participants rested within the laboratory throughout each trial, except from 0 – 1 h during the exercise trial where participants performed 60 min of treadmill running at ~70% of VO_2 max. Expired air samples were collected as described earlier to calculate the net energy expenditure of exercise. A test meal was provided at 2 h, consisting of a ham sandwich, banana, salted crisps and chocolate bar. The macronutrient composition of the meals was

63% carbohydrate, 9% protein and 28% fat. The energy content was 42 kJ per kg body mass (men 3167 (395) kJ; women 2599 (305) kJ).

Appetite perceptions and *ad libitum* buffet meals

Appetite perceptions (hunger, satisfaction, fullness and prospective food consumption) were assessed at baseline and every 30 min during both experiments using 100 mm visual analogue scales (11). An overall appetite rating was calculated for each time-point as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness (32). At 8 h during experiment one and 5 h during experiment two, participants were given 30 min access to a buffet meal from which they were free to select and consume food *ad libitum*. The buffet was set up identically before each meal with food being presented in excess of expected consumption. The items available were milk, three varieties of cereal, cereal bars, white bread, brown bread, ham, cheese, tuna, mayonnaise, butter, margarine, cookies, chocolate rolls, apples, oranges and bananas. Participants were told to eat until satisfied and that additional food was available if desired. Participants were not overtly aware that their food intake was being monitored with actual intake being deduced by experimenters covertly re-weighing leftover foods after *ad libitum* meals. Energy and macronutrient intake was determined using values provided by the food manufacturers. All meals were consumed in isolation so that social influence did not affect food selection. Water was available *ad libitum* throughout each trial.

Blood sampling and analysis

During the experimental trials, venous blood samples were collected via a cannula (Venflon, Becton Dickinson, Helsinborg, Sweden) inserted into an antecubital vein. Blood samples were collected at baseline, 2, 3, 4.75, 6, 7, 8 and 9 h in experiment one and baseline, 0.5, 1, 2, 2.5, 3, 4, 4.5, 5, 5.5, 6, and 7 h in experiment two. Plasma acylated ghrelin concentrations were measured from blood samples in both experiments and PYY_{3-36} was additionally measured in experiment one. Details on acylated ghrelin and PYY_{3-36} sample collection and processing have been described in-depth previously (7).

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 6.9 and 6.8% for acylated ghrelin and PYY_{3-36}, respectively.

Statistical analysis

Data was analysed using IBM SPSS statistics version 19 for Windows. Time-averaged area under the curve (AUC) values were calculated using the trapezoidal method. For experiment one, one-way repeated measures ANOVA was used to assess trial-based differences in energy intake at the *ad libitum* meal as well as AUC values for appetite, acylated ghrelin and PYY_{3-36}. For experiment two, independent samples t-tests were used to assess baseline differences between male and female participants. Mixed measures, two-way ANOVA (sex x trial) was used to assess differences in energy intake and AUC values for appetite and acylated ghrelin. Where significant main effects were found, post-hoc analysis was performed using Holm-Bonferonni correction for multiple comparisons. Statistical significance for this study was accepted as $P \leq 0.05$. 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.

Sample size calculations

The sample sizes employed within this study were deemed sufficient to detect a significant difference in energy intake between trials in experiment one and a significant difference in relative energy intake between sexes in experiment two. These variables were selected as the primary outcome measure for each experiment. The anticipated effect size for a difference in energy intake between trials for experiment one was based on previous findings from our laboratory using an identical experimental protocol in men (20). The anticipated effect size for a difference in relative energy intake between sexes for experiment two was based on the findings from previous research that employed similar methods to the present experiment (16). Based on these effect sizes and an alpha value of 5%, a sample size of 12 participants in experiment one would have > 95 % power to detect a difference in energy intake and 20 participants (10 men and 10 women) in experiment two would have > 87 % power to detect a difference in relative energy intake between sexes. All calculations were performed using G*power (9).

RESULTS

Experiment One

Participant characteristics and exercise responses

The physical characteristics of participants are described in Table 1. Participants rated 'low' for each trait within the TFEQ (cognitive restraint 7.8 (3.3); disinhibition 7.9 (3.2); hunger 6.9 (3.1). Participants completed the 90 min run at 8.6 (1.0) $km.h^{-1}$. This elicited an oxygen consumption equivalent to 70.2 (1.5) % of VO_2 max and a net energy expenditure of 3560 (382) kJ. The non-protein respiratory exchange ratio was 0.86 (0.04) which reflected a proportional contribution to energy provision of 54 (13) % carbohydrate and 46 (13) % fat.

Heart rate and rating of perceived exertion (RPE) were 175 (3) beats.min^{-1} and 13 (1), respectively.

Appetite and energy intake

Overall appetite ratings did not differ between trials at baseline (Ex-Def 71 (23); Food-Def 77 (12); Con 75 (16); $P = 0.536$). One-way ANOVA revealed higher appetite AUC in Food-Def than Ex-Def and Con across the 9 h trial ($P < 0.0005$; Figure 1 and 2). At the *ad libitum* buffet meal, total energy intake was significantly higher in Food-Def than Ex-Def and Control (Ex-Def 2774 (1682); Food-Def 3965 (1409); Control 2560 (1112) kJ; $P < 0.0005$). Similarly, energy intake from fat, protein and carbohydrate was significantly higher in Food-Def than Ex-Def and Control (all $P < 0.004$; data not presented).

Plasma acylated ghrelin and $PYY_{3\text{-}36}$ concentrations

Due to problems with venous cannulation acylated ghrelin and $PYY_{3\text{-}36}$ data is only available for 11 participants. Fasting plasma acylated ghrelin concentrations did not differ significantly between trials at baseline (Con 148 (100); Ex-Def 140 (86); Food-Def 148 (96) pg.mL^{-1}; $P = 0.422$). Acylated ghrelin concentrations were significantly higher in Food-Def and significantly lowest in Ex-Def across the 9 h trial ($P < 0.0005$; Figure 1 and 2). Fasting $PYY_{3\text{-}36}$ concentrations did not differ significantly between trials at baseline (Con 77 (39); Ex-Def 76 (34); Food-Def 77 (36) pg.mL^{-1}; $P = 0.989$). Time-averaged AUC for $PYY_{3\text{-}36}$ was significantly highest in Ex-Def and significantly lowest in Food-Def across the 9 h trial ($P < 0.0005$; Figure 1 and 2).

Experiment Two

Participant characteristics and exercise responses

The physical characteristics of the participants are described and contrasted (men versus women) in Table 1. There were no differences between men and women in their TFEQ scores for cognitive restraint (men: 6 (1); women: 8 (2)), disinhibition (men: 4 (1); women: 6 (1)) or hunger (men: 6 (1); women: 7 (1)). The 60 min run was completed at a significantly higher speed in men than women (men: 10.7 (0.7) $km.h^{-1}$; women: 8.4 (0.3) $km.h^{-1}$; P = 0.006). The run also generated a greater net energy expenditure in men than women (men: 3971 (200) kJ; women: 2536 (126) kJ; P < 0.0005). However, there was no difference in relative exercise intensity (70.9 (1.4) % and 73.3 (0.6) % of VO_2 max in men and women respectively; P = 0.130). There was a tendency for a lower heart rate in men than women (men: 163 (4) $beats.min^{-1}$; women: 174 (4) $beats.min^{-1}$; P = 0.068). Ratings of perceived exertion did not differ between sexes (13 (1) and 12 (0) in men and women respectively; P = 0.797).

Appetite and energy intake

Appetite did not differ by trial (exercise vs. Con) or sex at baseline (Female-Ex 61 (22); Female Con 65 (11); Male Ex 70 (12); Male Con 74 (11); all P > 0.05). Two-way ANOVA revealed main effects of trial (P = 0.05) and sex (P = 0.01) for AUC appetite ratings across the 7 h trial, with higher appetite ratings in men than women and in control compared with exercise (Figure 3).

Two-factor ANOVA revealed a main effect of sex for energy intake (P = 0.023) and carbohydrate intake (P = 0.013) during the *ad libitum* buffet meal, indicating greater consumption by men than women. Differences between sexes no longer remained after intakes were adjusted for lean body mass (both P ≥ 0.289). There was no effect of trial for energy or macronutrient intake and no differences between sexes for fat and protein intake (both P > 0.05; Table 2).

Two-factor ANOVA revealed a main effect of trial for relative energy intake (energy intake minus net energy expenditure of exercise) indicating lower relative energy intake in the exercise trial compared with control (Female Ex 442 (1711); Female Con 2916 (1510); Male Ex 1414 (2510); Male Con 4971 (2648) kJ; $P < 0.0005$). This resulted in a similar energy deficit for men and women in the exercise trial relative to control (men: 3557 (598); women: 2474 (406) kJ; $P = 0.152$).

Acylated ghrelin

Due to problems with venous cannulation, acylated ghrelin data is only available for 8 men and 8 women. Baseline values were not different between control and exercise trials ($P > 0.05$) but were significantly higher in women than men (Female Ex 155 (101); Female Con 178 (61); Male Ex 71 (31); Male Con 100 (56); $P = 0.018$). Two-way ANOVA revealed main effects of trial ($P = 0.004$) and sex ($P = 0.034$) for AUC acylated ghrelin concentrations across the 7 h trial, with higher concentrations in women than men and in control compared with exercise (Figure 4).

DISCUSSION

In recent years there has been an explosion of research examining the interaction between exercise and energy homeostasis. One area which has received widespread attention is the influence of exercise and associated changes in energy balance on gut hormones which have been identified as key regulators of appetite, energy intake and adiposity (21,30,31). To date, the majority of research within these areas has been conducted using male particpants meaning that much less is known regarding the interaction between acute exercise and food intake regulation in women. The findings of the present experiments demonstrate that women respond similarly to men with regards to short-term responses to energy deficits induced by exercise and food restriction. Specifically, in accordance with our previous results in male participants (20), in experiment one, our female sample demonstrated rapid and robust compensatory appetite, energy intake and appetite hormone responses (acylated ghrelin and PYY_{3-36}) to energy deficits induced by food restriction but not exercise. Additionally, in experiment two, both male and female participants exhibited suppressed appetite and circulating acylated ghrelin in response to exercise without any change in *ad libitum* energy intake being apparent. These data provide new information regarding short-term physiological and behavioural responses to energy deficits in women.

Experiment one showed that in women an acute energy deficit of ~3500 kJ robustly stimulated appetite and energy intake when induced via energy restriction but such compensatory responses did not occur when an equivalent deficit was induced by exercise. These outcomes are consistent with the findings from an identical previous study in men (20) and highlight the importance of oro-gastric mechanisms e.g. stomach distention and/or passage of nutrients through the gastrointestinal tract, for short-term appetite control in men and women (2,36). Such regulatory mechanisms are complemented by a network of appetite regulatory hormones, and the identification of higher circulating concentrations of acylated ghrelin, and lower PYY_{3-36} in response to energy restriction, is consistent with the known acute regulatory actions of these hormones (24,25). In contrast, within experiment one, exercise elicited reductions in circulating acylated ghrelin and elevations in PYY_{3-36} across the 9 h trial in our female sample. These responses are consistent with previous studies in men which have identified a potent capacity of exercise to perturb the circulating concentrations of these hormones in directions associated with a reduction in appetite (30). The mechanisms promoting such changes are unclear and were not investigated in the present experiments. It has been suggested that exercise-induced changes in sympathetic nervous system activity (3,38) and splanchnic blood flow (29,37) may be important, however additional work is needed to investigate this issue. As per our previous findings in males (20), the results from experiment one demonstrate the usefulness of exercise for weight management in women to minimise compensatory responses associated with energy deficits produced solely by dietary restriction. Additional research is now needed to determine the more prolonged impact of exercise and diet-related energy deficits on appetite and energy intake in men and women; research that will provide more tangible information for individuals concerned with weight management.

The second experiment of this paper demonstrated that an acute bout of exercise, performed at the same relative exercise intensity, decreased appetite ratings in men and women. Furthermore, this response was consistent with lower acylated ghrelin concentrations in both sexes and the absence of any compensatory increase in *ad libitum* energy intake. These findings are consistent with the suggestion that men and women do not differ in their physiological and behavioural responses to exercise (5) and this notion is supported by previous data, albeit with a very brief period of observation after exercise (16). Our findings therefore add to the literature by demonstrating that acute responses to exercise do not differ between men and women over a prolonged duration within the laboratory.

In contrast to the present results, previous research has shown that appetite is not suppressed in women during exercise (18,22,23). Furthermore, Larson-Meyer et al. (23) observed an increase in circulating acylated ghrelin in response to acute exercise; contrasting the suppression reported in the present paper. The discrepant findings with regards to appetite may be related to exercise intensity with the intensity in the present studies being much greater (70% of VO_2 max) than that employed by Hopkins et al (18) (~50% of VO_2 max). Training status and familiarity with exercise also moderate exercise-related appetite responses (26,27) and the lack of influence of exercise on appetite in the studies of King et al. (22) and Larson-Meyer et al. (23) may be because their participants were regularly active and particularly familiar with the mode of exercise employed. An increase in circulating acylated ghrelin in response to exercise (23) contrasts the present findings and the bulk of the literature which has studied men (30). Regression to the mean may have been a confounding factor in the study of Larson-Meyer et al. (23) however. Furthermore, differences in the analytical techniques utilised between studies may also be influential. Nonethless, despite these noted discrepancies, *ad libitum* energy intake remained unchanged in each of the aforementioned studies. Thus, as seen in men, single sessions of exercise do not appear to influence energy intake in women.

Although we found no differences between sexes in compensatory responses to exercise, females participants exhibited significantly higher plasma acylated ghrelin concentrations across main trials compared with men – a finding which has been reported previously (13). Despite this disparity, appetite ratings were paradoxically higher in men than women across main trials. This difference may highlight the importance of relative changes in gut hormone concentrations, rather than absolute circulating levels which may markedly differ between individuals. The similar acylated ghrelin response to exercise in both sexes may therefore underpin the comparable appetite and energy intake responses observed. Given that acylated ghrelin and $PYY_{3\text{-}36}$ function within a network of other key appetite regulatory peptides (17), additional research is needed to characterise the impact of the present interventions on glucagon-like-peptide-1, oxyntomodulin, pancreatic polypeptide and leptin in men compared with women.

The higher appetite ratings and food intake seen in men in experiment two supports the concept that lean body mass is the primary determinant of tonic appetite ratings and energy intake (1). This theory is further supported by our finding that energy intake during *ad libitum* feeding did not differ between sexes when expressed per kilogram of lean body mass. Although acylated ghrelin may in part mediate the episodic changes in appetite observed in the present study, the lower tonic concentrations observed in men suggests that lean body mass may influence appetite and energy intake through an alternative mechanism. Recent evidence suggests that resting metabolic rate may be important in this regard (1).

Our findings provide a comparative insight into the short-term appetite, energy intake and gut hormone responses to acute energy deficits in women compared with men. In accordance with the recent findings of Caudwell et al. (5), these new data support the perspective that men and women do not exhibit different physiological or behavioural compensatory responses to energy deficits (induced by exercise or food-restriction); at least during the actual day when an energy deficit is imposed. Our findings therefore support the importance of exercise for weight management in women however these data must be considered in light of certain limitations. Firstly, both experiment one and two were powered to detect changes in food intake and it is possible that subtle effects of the present interventions on appetite and gut hormones may not have been detected. Secondly, the implementation of prolonged and strenuous exercise protocols, completed by recreationally active individuals, may limit the generalisability of the findings i.e. to those who are less active or less fit. The arduous exercise undertaken in the present studies may therefore not be achievable by many seeking to commence a weight loss program and additional work is needed with overweight and/or obese participants.

In conclusion, the experiments presented in this paper have provided evidence that appetite, energy intake and gut hormone responses to acute energy deficits do not differ between men and women. These data support the importance of exercise for weight management in women to reduce the compensatory responses to energy deficits achieved solely via food restriction.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest. The results of the present study do not constitute endorsement by ACSM.

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FIGURE CAPTIONS

Figure 1. Time-averaged appetite (a), circulating acylated ghrelin (b) and peptide YY_{3-36} (c) AUC for each 9 h trial. *Food-Def significantly different from Ex-Def and control; † Ex-Def significantly different from Food-Def and control (experiment one – female participants only). Values are mean (SEM), N = 12 for appetite and 11 for acylated ghrelin and peptide $YY_{3-36.}$

Figure 2. Appetite (a), circulating acylated ghrelin (b) and peptide YY_{3-36} (c) concentrations across the Con (▼), Ex-Def (●) and Food-Def (○) trials (experiment one – female participants only). Hatched shaded rectangles indicate standardised test meals, lightly shaded rectangle indicates exercise, black rectangle indicates *ad libitum* meal. Values are mean (SEM), N = 12 for appetite and 11 for acylated ghrelin and peptide $YY_{3-36.}$

Figure 3. (a) Appetite ratings in Male Con (○), Male Ex (●), Female Con (▽) and Female Ex (▼) (experiment two – male and female participants). Hatched shaded rectangles indicate standardised test meal, lightly shaded rectangle indicates exercise, black rectangle indicates *ad libitum* meal. (b) Time-averaged appetite AUC for each 7 h trial. ‡ Males significant different than females. § Control significantly different than exercise. Values are mean (SEM). Females N=10; males N=10.

Figure 4. (a) Plasma acylated ghrelin concentrations in Male Con (○), Male Ex (●), Female Con (▽) and Female Ex (▼) (experiment two – male and female participants). Hatched shaded rectangles indicate standardised test meal, lightly shaded rectangle indicates exercise, black rectangle indicates *ad libitum* meal. (b) Time-averaged acylated ghrelin AUC for each

7 h trial. ¶ Females significantly different than males. § Control significantly different than exercise. Values are mean (SEM). Females N=8; males N=8.

Table 1: Participant characteristics in experiment one and two

	Experiment 1 (Females)	**Experiment 2 (Females)**	**(Males)**
Participant number (*n*)	12[‡]	10[†]	10[†]
Age (y)	22.4 (2.1)	22.3 (2.5)	22.6 (3.8)
Height (cm)	165.6 (5.4)	166.6 (5.4)	180.5 (6.2)*
Body mass (kg)	60.4 (4.2)	61.9 (7.3)	75.4 (9.4)*
BMI (kg/m^2)	22.0 (1.1)	22.3 (2.32)	23.1 (2.1)
Body Fat (%)	24.1 (2.8)	22.4 (5.5)	10.1 (4.2)*
Lean mass (kg)	45.9 (3.7)	47.4 (1.4)	67.5 (3.3)*
VO2 max (mL/kg/min)	50.4 (4.3)	48.8 (6.1)	66.1 (9.2)*

*significantly different between males and females ($P < 0.005$)

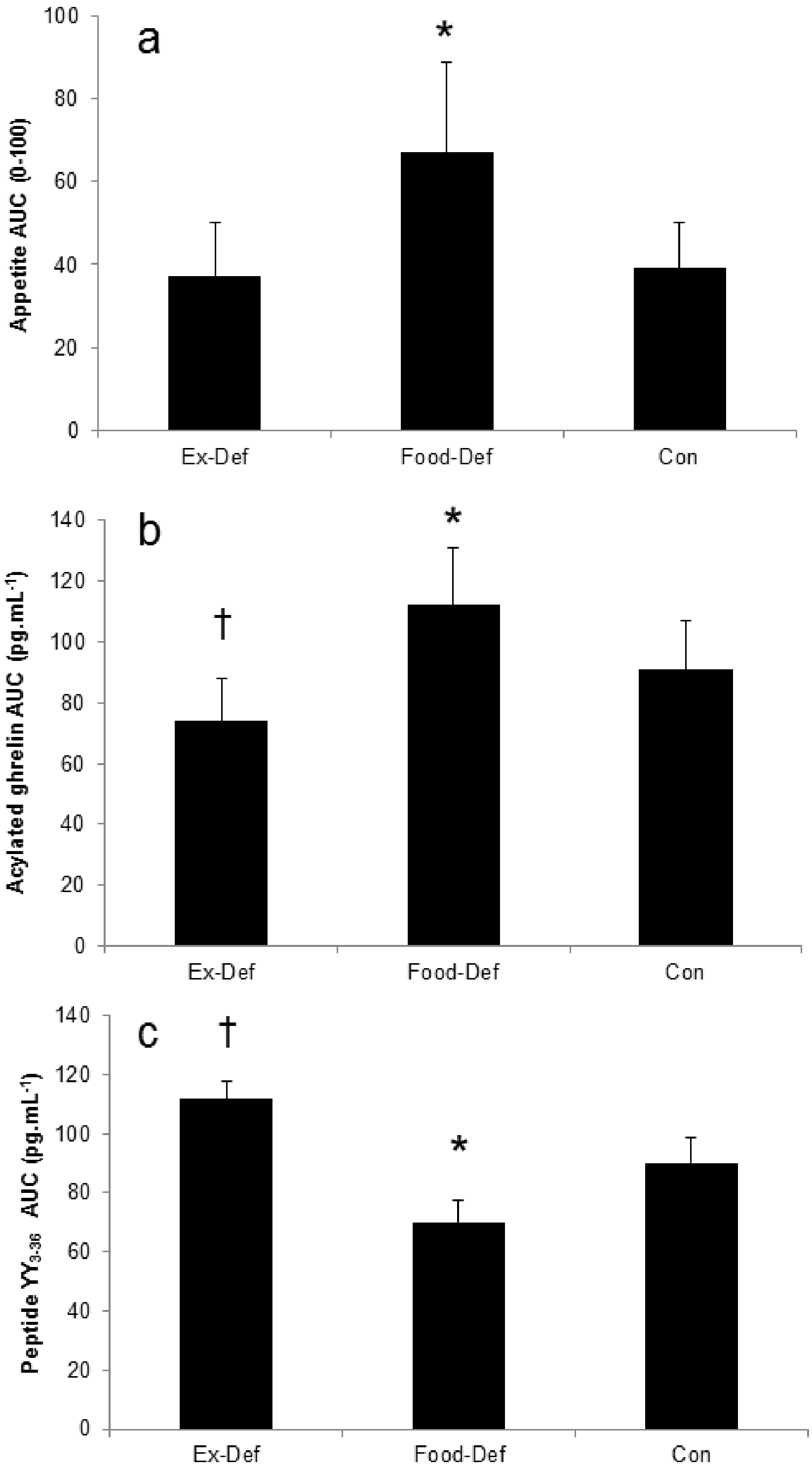
[‡]acylated ghrelin and $PYY_{3\text{-}36}$ data available for 11 participants

[†] acylated ghrelin data available for 8 participants

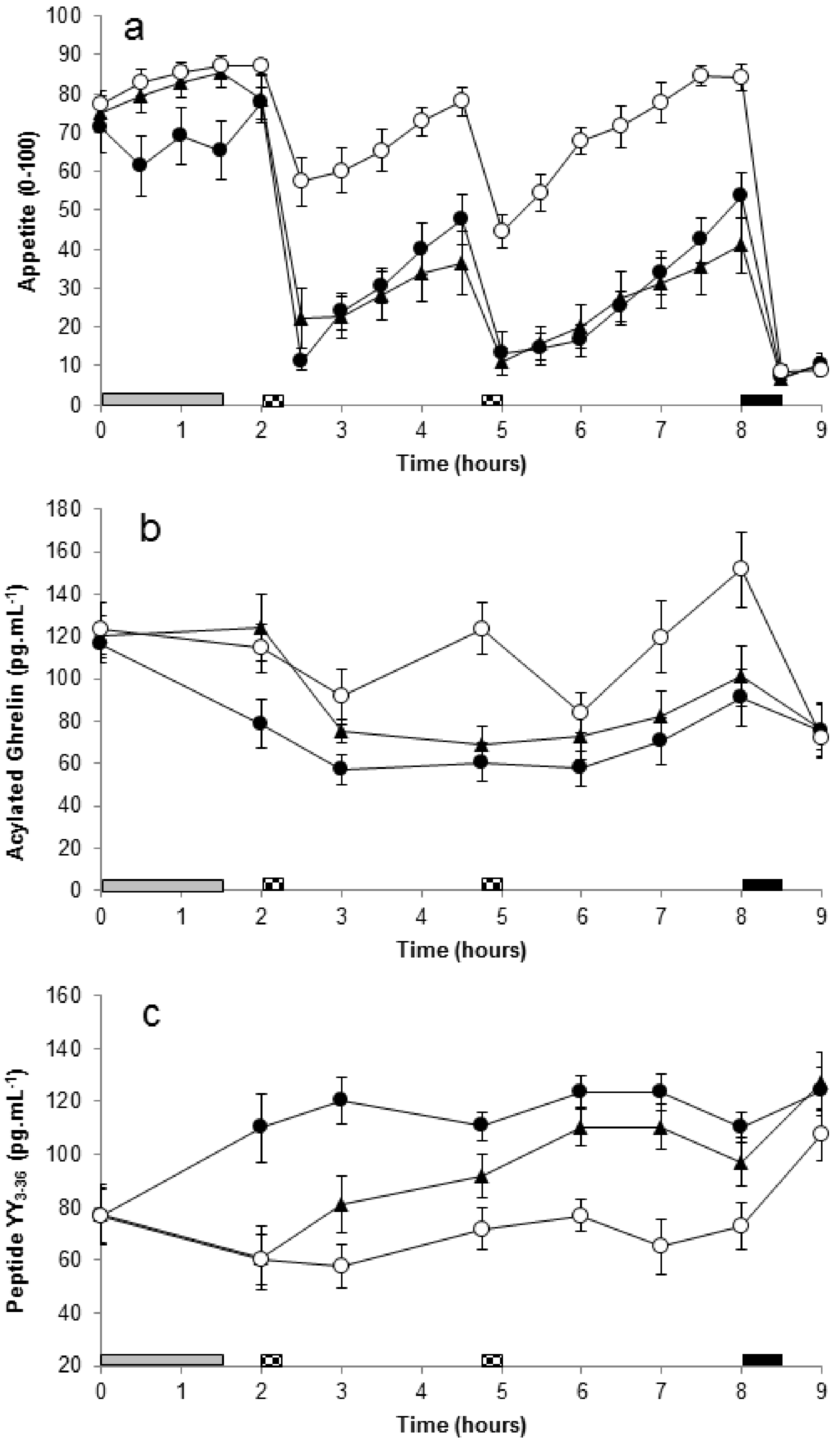
Table 2. Energy and macronutrient intakes of men and women during the buffet meal in the control and exercise trials.

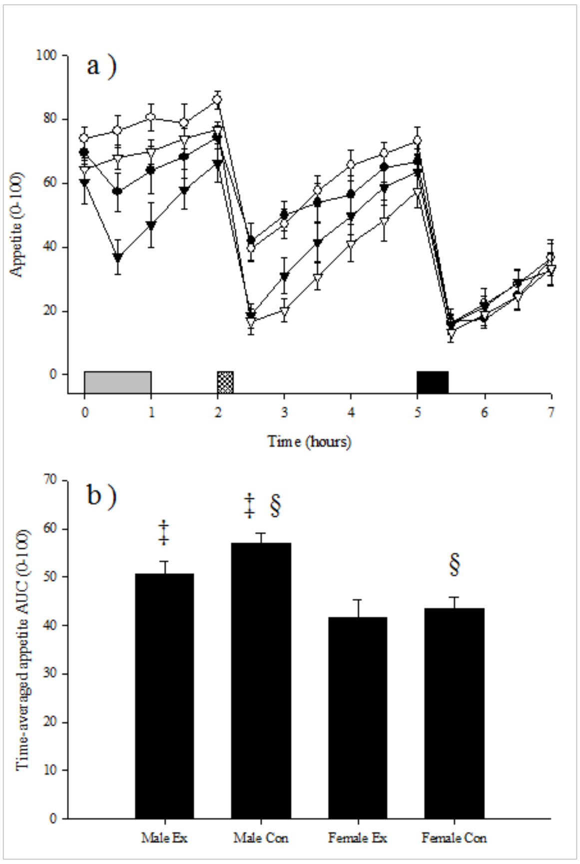
	Control		Exercise	
	Men	Women	Men	Women
Fat (kJ)	355 ± 274	175 ± 142	348 ± 245	168 ± 142
Fat (kJ.kg lean mass^{-1})	5 ± 5	4 ± 3	5 ± 3	4 ± 3
Carbohydrate (kJ)‡	680 ± 318	434 ± 174	788 ± 322	446 ± 201
Carbohydrate (kJ.kg lean mass^{-1})	10 ± 6	9 ± 4	12 ± 6	10 ± 5
Protein (kJ)	148 ± 111	87 ± 75	149 ± 100	95 ± 68
Protein (kJ.kg lean mass^{-1})	2 ± 1	2 ± 1	2 ± 1	2 ± 1
Energy intake (kJ)‡	4971 ± 2644	2916 ± 1506	5385 ± 2423	2979 ± 1586
Energy intake (kJ.kg lean mass^{-1})	75 ± 38	63 ± 25	84 ± 38	63 ± 38

Values are mean (SD). Females n=10; males n=10. ‡Significantly higher in men than women ($P < 0.05$).

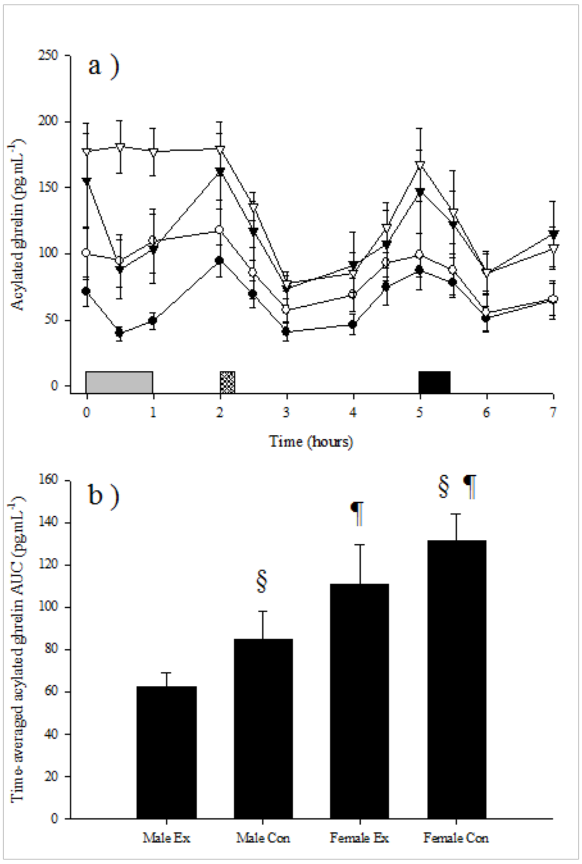


a
Appetite AUC (0-100)
100
80
60
40
20
0
*
Ex-Def
Food-Def
Con
b
Acylated ghrelin AUC (pg.mL-1)
140
120
100
80
60
40
20
0
*
†
Ex-Def
Food-Def
Con
c
Peptide YY3-36 AUC (pg.mL-1)
140
120
100
80
60
40
20
0
†
*
Ex-Def
Food-Def
Con


a
Appetite (0-100)
Time (hours)
b
Acylated Ghrelin (pg.mL-1)
Time (hours)
c
Peptide YY3-36 (pg.mL-1)
Time (hours)



a)
Appetite (0-100)
100
80
60
40
20
0
0
1
2
3
4
5
6
7
Time (hours)
b)
Time-averaged appetite AUC (0-100)
70
60
50
40
30
20
10
0
‡
‡ §
§
Male Ex
Male Con
Female Ex
Female Con



a)
Acylated ghrelin (pg.mL-1)
Time (hours)
b)
Time-averaged acylated ghrelin AUC (pg.mL-1)
Male Ex
Male Con
Female Ex
Female Con
§
¶
§ ¶