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

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26

27 **ABSTRACT**

28 **Purpose:** To explore whether compensatory responses to acute energy deficits induced by  
29 exercise or diet differ by sex. **Methods:** In experiment one, twelve healthy women completed  
30 three 9 h trials (control, exercise-induced (Ex-Def) and food restriction induced energy deficit  
31 (Food-Def)) with identical energy deficits being imposed in the Ex-Def (90 min run, ~70% of  
32 VO<sub>2</sub> max) and Food-Def trials. In experiment two, 10 men and 10 women completed two 7 h  
33 trials (control and exercise). Sixty min of running (~70% of VO<sub>2</sub> max) was performed at the  
34 beginning of the exercise trial. Participants rested throughout the remainder of the exercise  
35 trial and during the control trial. Appetite ratings, plasma concentrations of gut hormones and  
36 *ad libitum* energy intake were assessed during main trials. **Results:** In experiment one, an  
37 energy deficit of ~3500 kJ induced via food restriction increased appetite and food intake.  
38 These changes corresponded with heightened concentrations of plasma acylated ghrelin and  
39 lower peptide YY<sub>3-36</sub>. None of these compensatory responses were apparent when an  
40 equivalent energy deficit was induced by exercise. In experiment two, appetite ratings and  
41 plasma acylated ghrelin concentrations were lower in exercise than control but energy intake  
42 did not differ between trials. The appetite, acylated ghrelin and energy intake response to  
43 exercise did not differ between men and women. **Conclusions:** Women exhibit compensatory  
44 appetite, gut hormone and food intake responses to acute energy restriction but not in  
45 response to an acute bout of exercise. Additionally, men and women appear to exhibit similar  
46 acylated ghrelin and PYY<sub>3-36</sub> responses to exercise-induced energy deficits. These findings  
47 advance understanding regarding the interaction between exercise and energy homeostasis in  
48 women.

49

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

52 **INTRODUCTION**

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

68

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

77 Over the past decade our laboratory has conducted many acute experimental trials seeking to  
78 enhance understanding concerning the short-term regulation of appetite and energy balance  
79 (21,31). In a sample of male participants, we recently demonstrated that the induction of an  
80 acute energy deficit by food restriction elicited a rapid and robust compensatory appetite, gut  
81 hormone (acylated ghrelin and PYY<sub>3-36</sub>) and energy intake response whilst the same energy  
82 deficit imposed by exercise had no effect (20). These findings suggest that the method by  
83 which an energy deficit is imposed has a marked impact on the subsequent physiological and  
84 behavioural response. It is currently unknown whether women exhibit the same acute  
85 responses to exercise and food restriction as men. This information has important  
86 implications regarding the utility of lifestyle therapies to assist weight control in women.

87

88 Within this report we describe the findings from two acute experimental studies which sought  
89 to provide new information regarding the short-term appetite, food intake and appetite  
90 hormone responses to exercise and food-induced energy deficits in men and women. In  
91 experiment one; we compared the appetite, energy intake, acylated ghrelin and PYY<sub>3-36</sub>  
92 responses to an equivalent energy deficit induced by exercise or energy restriction in women.  
93 In experiment two, we directly compared appetite, food intake and circulating acylated  
94 ghrelin responses to an exercise-induced energy deficit in men verses women. Our findings  
95 identify a high degree of similarity in the acute response to energy deficits in men and  
96 women.

97

## 98 **METHODS**

### 99 **Experimental protocol**

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

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

109

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

118

### 119 **Preliminary trials**

120 In order to determine the running speed required to elicit 70% of maximum oxygen uptake  
121 ( $\text{VO}_2 \text{ max}$ ) for each individual, participants completed a preliminary trial before the main  
122 trials for each experiment. This consisted of a submaximal running test and a  $\text{VO}_2 \text{ max}$  test  
123 on a motorised treadmill (34). Anthropometric measurements and study questionnaires e.g.  
124 Three Factor Eating Questionnaire (TFEQ) (33) was also taken/completed at this time. At this  
125 visit, participants also verbally confirmed acceptability of the test meals and *ad libitum* meals  
126 subsequently to be provided during main experimental trials.

127

128 In experiment one a second preliminary trial was completed to determine the net energy cost  
129 of exercise which was needed to calculate food provision in the main trials and to enable trial  
130 randomisation in advance. During this session participants ran for 90 min at 70% of VO<sub>2</sub> max  
131 with expired air samples being collected into Douglas bags at 15 min intervals to calculate  
132 energy expenditure using the equations provided by Frayn (12).

133

### 134 **Experiment One**

135 Twelve female participants performed three 9 h experimental trials (control (Con)), exercise-  
136 induced energy deficit (Ex-Def) and diet-induced energy deficit (Food-Def)) separated by  
137 one-week in a randomised counterbalanced design. To ensure standardisation of menstrual  
138 phase, participants' first main trial was undertaken at the beginning of their follicular phase  
139 with their second trial occurring one-week later. Participants' third main trial was  
140 subsequently undertaken at the beginning of their next cycle approximately four weeks later.  
141 Participants rested within the laboratory throughout all trials with participants being  
142 permitted to read, work at a computer or watch DVDs which had been screened to ensure that  
143 there was no overt emphasis on food and drink. The exception to this occurred at 0-1.5 h  
144 during Ex-Def where participants performed 90 min of treadmill running at ~70% of VO<sub>2</sub>  
145 max (identical to that performed during the preliminary trial). Resting expired air samples  
146 were collected from 0 – 1.5 h during the Con and Food-Def trials to calculate the net energy  
147 expenditure of exercise (gross energy expenditure of exercise minus energy expenditure at  
148 rest) (12).

149

150 Identical test meals were provided at 2 h (breakfast) and 4.75 h (lunch) and were each  
151 consumed within 15 min. The meals consisted of a tuna and mayonnaise sandwich, salted

152 crisps, chocolate muffin and green apple. The macronutrient composition of the meal was  
153 47% carbohydrate, 18% protein and 35% fat. The energy content of the test meals was  
154 identical in Con and Ex-Def (2778 (109) kJ) with each meal providing 35% of participants'  
155 estimated daily energy needs for a sedentary day. This calculation was based upon an  
156 estimation of each participant's daily energy needs which was determined using a validated  
157 equation for resting metabolic rate (28) that was multiplied by an activity factor (1.4) deemed  
158 appropriate for a sedentary day (10). In Food-Def, the energy content of the test meals was  
159 reduced (1025 (159) kJ) by deducting the net energy expenditure of exercise from the energy  
160 provided at the test meals during Con and Ex-Def. This energy deficit was individually  
161 prescribed based on the exercise energy expenditure data derived from the preliminary trials  
162 and the total amount of energy deducted was divided equally between breakfast and lunch.  
163 Therefore, equivalent energy deficits were induced in Ex-Def and Food-Def relative to Con.  
164 The macronutrient percentage of the test meals was identical across main trials i.e. only the  
165 meal energy content was altered in the Food-Def trial.

166

## 167 **Experiment Two**

168 Ten female and 10 male participants performed two 7 h experimental trials (exercise and  
169 control) separated by one week in a randomised counterbalanced design. Female participants  
170 completed both main trials during the follicular phase (days 1 – 11) of their menstrual cycle.  
171 Participants rested within the laboratory throughout each trial, except from 0 – 1 h during the  
172 exercise trial where participants performed 60 min of treadmill running at ~70% of  $\text{VO}_2$  max.  
173 Expired air samples were collected as described earlier to calculate the net energy  
174 expenditure of exercise. A test meal was provided at 2 h, consisting of a ham sandwich,  
175 banana, salted crisps and chocolate bar. The macronutrient composition of the meals was



176 63% carbohydrate, 9% protein and 28% fat. The energy content was 42 kJ per kg body mass  
177 (men 3167 (395) kJ; women 2599 (305) kJ).

178

### 179 **Appetite perceptions and *ad libitum* buffet meals**

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

196

### 197 **Blood sampling and analysis**

198 During the experimental trials, venous blood samples were collected via a cannula (Venflon,  
199 Becton Dickinson, Helsinborg, Sweden) inserted into an antecubital vein. Blood samples  
200 were collected at baseline, 2, 3, 4.75, 6, 7, 8 and 9 h in experiment one and baseline, 0.5, 1, 2,

201 2.5, 3, 4, 4.5, 5, 5.5, 6, and 7 h in experiment two. Plasma acylated ghrelin concentrations  
202 were measured from blood samples in both experiments and PYY<sub>3-36</sub> was additionally  
203 measured in experiment one. Details on acylated ghrelin and PYY<sub>3-36</sub> sample collection and  
204 processing have been described in-depth previously (7).

205

206 A commercially available enzyme immunoassay was used to determine plasma  
207 concentrations of acylated ghrelin (SPI BIO, Montigny le Bretonneux, France). Plasma  
208 concentrations of PYY<sub>3-36</sub> were determined using a commercially available  
209 radioimmunoassay (Millipore, Watford, UK). To eliminate interassay variation, samples from  
210 each participant were analysed in the same run. The within batch coefficient of variation for  
211 the assays were 6.9 and 6.8% for acylated ghrelin and PYY<sub>3-36</sub>, respectively.

212

### 213 **Statistical analysis**

214 Data was analysed using IBM SPSS statistics version 19 for Windows. Time-averaged area  
215 under the curve (AUC) values were calculated using the trapezoidal method. For experiment  
216 one, one-way repeated measures ANOVA was used to assess trial-based differences in  
217 energy intake at the *ad libitum* meal as well as AUC values for appetite, acylated ghrelin and  
218 PYY<sub>3-36</sub>. For experiment two, independent samples t-tests were used to assess baseline  
219 differences between male and female participants. Mixed measures, two-way ANOVA (sex x  
220 trial) was used to assess differences in energy intake and AUC values for appetite and  
221 acylated ghrelin. Where significant main effects were found, post-hoc analysis was  
222 performed using Holm-Bonferonni correction for multiple comparisons. Statistical  
223 significance for this study was accepted as  $P \leq 0.05$ . Results in text and tables are presented  
224 as mean (SD). Graphical representations of results are presented as mean (SEM) to avoid  
225 distortion of the graphs.

## 226 **Sample size calculations**

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

240

## 241 **RESULTS**

### 242 **Experiment One**

#### 243 **Participant characteristics and exercise responses**

244 The physical characteristics of participants are described in Table 1. Participants rated ‘low’  
245 for each trait within the TFEQ (cognitive restraint 7.8 (3.3); disinhibition 7.9 (3.2); hunger  
246 6.9 (3.1). Participants completed the 90 min run at 8.6 (1.0) km.h<sup>-1</sup>. This elicited an oxygen  
247 consumption equivalent to 70.2 (1.5) % of VO<sub>2</sub> max and a net energy expenditure of 3560  
248 (382) kJ. The non-protein respiratory exchange ratio was 0.86 (0.04) which reflected a  
249 proportional contribution to energy provision of 54 (13) % carbohydrate and 46 (13) % fat.

250 Heart rate and rating of perceived exertion (RPE) were 175 (3) beats.min<sup>-1</sup> and 13 (1),  
251 respectively.

252

### 253 **Appetite and energy intake**

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

261

### 262 **Plasma acylated ghrelin and PYY<sub>3-36</sub> concentrations**

263 Due to problems with venous cannulation acylated ghrelin and PYY<sub>3-36</sub> data is only available  
264 for 11 participants. Fasting plasma acylated ghrelin concentrations did not differ significantly  
265 between trials at baseline (Con 148 (100); Ex-Def 140 (86); Food-Def 148 (96) pg.mL<sup>-1</sup>; P =  
266 0.422). Acylated ghrelin concentrations were significantly higher in Food-Def and  
267 significantly lowest in Ex-Def across the 9 h trial (P < 0.0005; Figure 1 and 2). Fasting PYY<sub>3-</sub>  
268 <sub>36</sub> concentrations did not differ significantly between trials at baseline (Con 77 (39); Ex-Def  
269 76 (34); Food-Def 77 (36) pg.mL<sup>-1</sup>; P = 0.989). Time-averaged AUC for PYY<sub>3-36</sub> was  
270 significantly highest in Ex-Def and significantly lowest in Food-Def across the 9 h trial (P <  
271 0.0005; Figure 1 and 2).

272

273

274

## 275 **Experiment Two**

### 276 **Participant characteristics and exercise responses**

277 The physical characteristics of the participants are described and contrasted (men versus  
278 women) in Table 1. There were no differences between men and women in their TFEQ scores  
279 for cognitive restraint (men: 6 (1); women: 8 (2)), disinhibition (men: 4 (1); women: 6 (1)) or  
280 hunger (men: 6 (1); women: 7 (1)). The 60 min run was completed at a significantly higher  
281 speed in men than women (men: 10.7 (0.7) km.h<sup>-1</sup>; women: 8.4 (0.3) km.h<sup>-1</sup>; P = 0.006). The  
282 run also generated a greater net energy expenditure in men than women (men: 3971 (200) kJ;  
283 women: 2536 (126) kJ; P < 0.0005). However, there was no difference in relative exercise  
284 intensity (70.9 (1.4) % and 73.3 (0.6) % of VO<sub>2</sub> max in men and women respectively; P =  
285 0.130). There was a tendency for a lower heart rate in men than women (men: 163 (4)  
286 beats.min<sup>-1</sup>; women: 174 (4) beats.min<sup>-1</sup>; P = 0.068). Ratings of perceived exertion did not  
287 differ between sexes (13 (1) and 12 (0) in men and women respectively; P = 0.797).

288

### 289 **Appetite and energy intake**

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

295

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

300 energy or macronutrient intake and no differences between sexes for fat and protein intake  
301 (both  $P > 0.05$ ; Table 2).

302

303 Two-factor ANOVA revealed a main effect of trial for relative energy intake (energy intake  
304 minus net energy expenditure of exercise) indicating lower relative energy intake in the  
305 exercise trial compared with control (Female Ex 442 (1711); Female Con 2916 (1510); Male  
306 Ex 1414 (2510); Male Con 4971 (2648) kJ;  $P < 0.0005$ ). This resulted in a similar energy  
307 deficit for men and women in the exercise trial relative to control (men: 3557 (598); women:  
308 2474 (406) kJ;  $P = 0.152$ ).

309

### 310 **Acylated ghrelin**

311 Due to problems with venous cannulation, acylated ghrelin data is only available for 8 men  
312 and 8 women. Baseline values were not different between control and exercise trials ( $P >$   
313  $0.05$ ) but were significantly higher in women than men (Female Ex 155 (101); Female Con  
314 178 (61); Male Ex 71 (31); Male Con 100 (56);  $P = 0.018$ ). Two-way ANOVA revealed main  
315 effects of trial ( $P = 0.004$ ) and sex ( $P = 0.034$ ) for AUC acylated ghrelin concentrations  
316 across the 7 h trial, with higher concentrations in women than men and in control compared  
317 with exercise (Figure 4).

318

## 319 **DISCUSSION**

320 In recent years there has been an explosion of research examining the interaction between  
321 exercise and energy homeostasis. One area which has received widespread attention is the  
322 influence of exercise and associated changes in energy balance on gut hormones which have  
323 been identified as key regulators of appetite, energy intake and adiposity (21,30,31). To date,  
324 the majority of research within these areas has been conducted using male participants

325 meaning that much less is known regarding the interaction between acute exercise and food  
326 intake regulation in women. The findings of the present experiments demonstrate that women  
327 respond similarly to men with regards to short-term responses to energy deficits induced by  
328 exercise and food restriction. Specifically, in accordance with our previous results in male  
329 participants (20), in experiment one, our female sample demonstrated rapid and robust  
330 compensatory appetite, energy intake and appetite hormone responses (acylated ghrelin and  
331 PYY<sub>3-36</sub>) to energy deficits induced by food restriction but not exercise. Additionally, in  
332 experiment two, both male and female participants exhibited suppressed appetite and  
333 circulating acylated ghrelin in response to exercise without any change in *ad libitum* energy  
334 intake being apparent. These data provide new information regarding short-term  
335 physiological and behavioural responses to energy deficits in women.

336

337 Experiment one showed that in women an acute energy deficit of ~3500 kJ robustly  
338 stimulated appetite and energy intake when induced via energy restriction but such  
339 compensatory responses did not occur when an equivalent deficit was induced by exercise.  
340 These outcomes are consistent with the findings from an identical previous study in men (20)  
341 and highlight the importance of oro-gastric mechanisms e.g. stomach distention and/or  
342 passage of nutrients through the gastrointestinal tract, for short-term appetite control in men  
343 and women (2,36). Such regulatory mechanisms are complemented by a network of appetite  
344 regulatory hormones, and the identification of higher circulating concentrations of acylated  
345 ghrelin, and lower PYY<sub>3-36</sub> in response to energy restriction, is consistent with the known  
346 acute regulatory actions of these hormones (24,25). In contrast, within experiment one,  
347 exercise elicited reductions in circulating acylated ghrelin and elevations in PYY<sub>3-36</sub> across  
348 the 9 h trial in our female sample. These responses are consistent with previous studies in  
349 men which have identified a potent capacity of exercise to perturb the circulating

350 concentrations of these hormones in directions associated with a reduction in appetite (30).  
351 The mechanisms promoting such changes are unclear and were not investigated in the present  
352 experiments. It has been suggested that exercise-induced changes in sympathetic nervous  
353 system activity (3,38) and splanchnic blood flow (29,37) may be important, however  
354 additional work is needed to investigate this issue. As per our previous findings in males (20),  
355 the results from experiment one demonstrate the usefulness of exercise for weight  
356 management in women to minimise compensatory responses associated with energy deficits  
357 produced solely by dietary restriction. Additional research is now needed to determine the  
358 more prolonged impact of exercise and diet-related energy deficits on appetite and energy  
359 intake in men and women; research that will provide more tangible information for  
360 individuals concerned with weight management.

361

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

371

372 In contrast to the present results, previous research has shown that appetite is not suppressed  
373 in women during exercise (18,22,23). Furthermore, Larson-Meyer et al. (23) observed an  
374 increase in circulating acylated ghrelin in response to acute exercise; contrasting the



375 suppression reported in the present paper. The discrepant findings with regards to appetite  
376 may be related to exercise intensity with the intensity in the present studies being much  
377 greater (70% of VO<sub>2</sub> max) than that employed by Hopkins et al (18) (~50% of VO<sub>2</sub> max).  
378 Training status and familiarity with exercise also moderate exercise-related appetite  
379 responses (26,27) and the lack of influence of exercise on appetite in the studies of King et al.  
380 (22) and Larson-Meyer et al. (23) may be because their participants were regularly active and  
381 particularly familiar with the mode of exercise employed. An increase in circulating acylated  
382 ghrelin in response to exercise (23) contrasts the present findings and the bulk of the  
383 literature which has studied men (30). Regression to the mean may have been a confounding  
384 factor in the study of Larson-Meyer et al. (23) however. Furthermore, differences in the  
385 analytical techniques utilised between studies may also be influential. Nonetheless, despite  
386 these noted discrepancies, *ad libitum* energy intake remained unchanged in each of the  
387 aforementioned studies. Thus, as seen in men, single sessions of exercise do not appear to  
388 influence energy intake in women.

389

390 Although we found no differences between sexes in compensatory responses to exercise,  
391 females participants exhibited significantly higher plasma acylated ghrelin concentrations  
392 across main trials compared with men – a finding which has been reported previously (13).  
393 Despite this disparity, appetite ratings were paradoxically higher in men than women across  
394 main trials. This difference may highlight the importance of relative changes in gut hormone  
395 concentrations, rather than absolute circulating levels which may markedly differ between  
396 individuals. The similar acylated ghrelin response to exercise in both sexes may therefore  
397 underpin the comparable appetite and energy intake responses observed. Given that acylated  
398 ghrelin and PYY<sub>3-36</sub> function within a network of other key appetite regulatory peptides (17),  
399 additional research is needed to characterise the impact of the present interventions on

400 glucagon-like-peptide-1, oxyntomodulin, pancreatic polypeptide and leptin in men compared  
401 with women.

402

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

411

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

425 to commence a weight loss program and additional work is needed with overweight and/or  
426 obese participants.

427

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

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439

440 **CONFLICT OF INTEREST**

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

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596 Table 1: Participant characteristics in experiment one and two

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

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

598 <sup>‡</sup>acylated ghrelin and PYY<sub>3-36</sub> data available for 11 participants

599 <sup>†</sup> acylated ghrelin data available for 8 participants

600

601

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

	Control		Exercise	
	Men	Women	Men	Women
Fat (kJ)	355 ± 274	175 ± 142	348 ± 245	168 ± 142
Fat (kJ.kg lean mass <sup>-1</sup> ) <sub>1)</sub>	5 ± 5	4 ± 3	5 ± 3	4 ± 3
Carbohydrate (kJ)‡	680 ± 318	434 ± 174	788 ± 322	446 ± 201
Carbohydrate (kJ.kg lean mass <sup>-1</sup> )	10 ± 6	9 ± 4	12 ± 6	10 ± 5
Protein (kJ)	148 ± 111	87 ± 75	149 ± 100	95 ± 68
Protein (kJ.kg lean mass <sup>-1</sup> )	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 <sup>-1</sup> )	75 ± 38	63 ± 25	84 ± 38	63 ± 38

604 Values are mean (SD). Females n=10; males n=10. ‡Significantly higher in men than women (P <  
 605 0.05).

606

607 **FIGURE CAPTIONS**

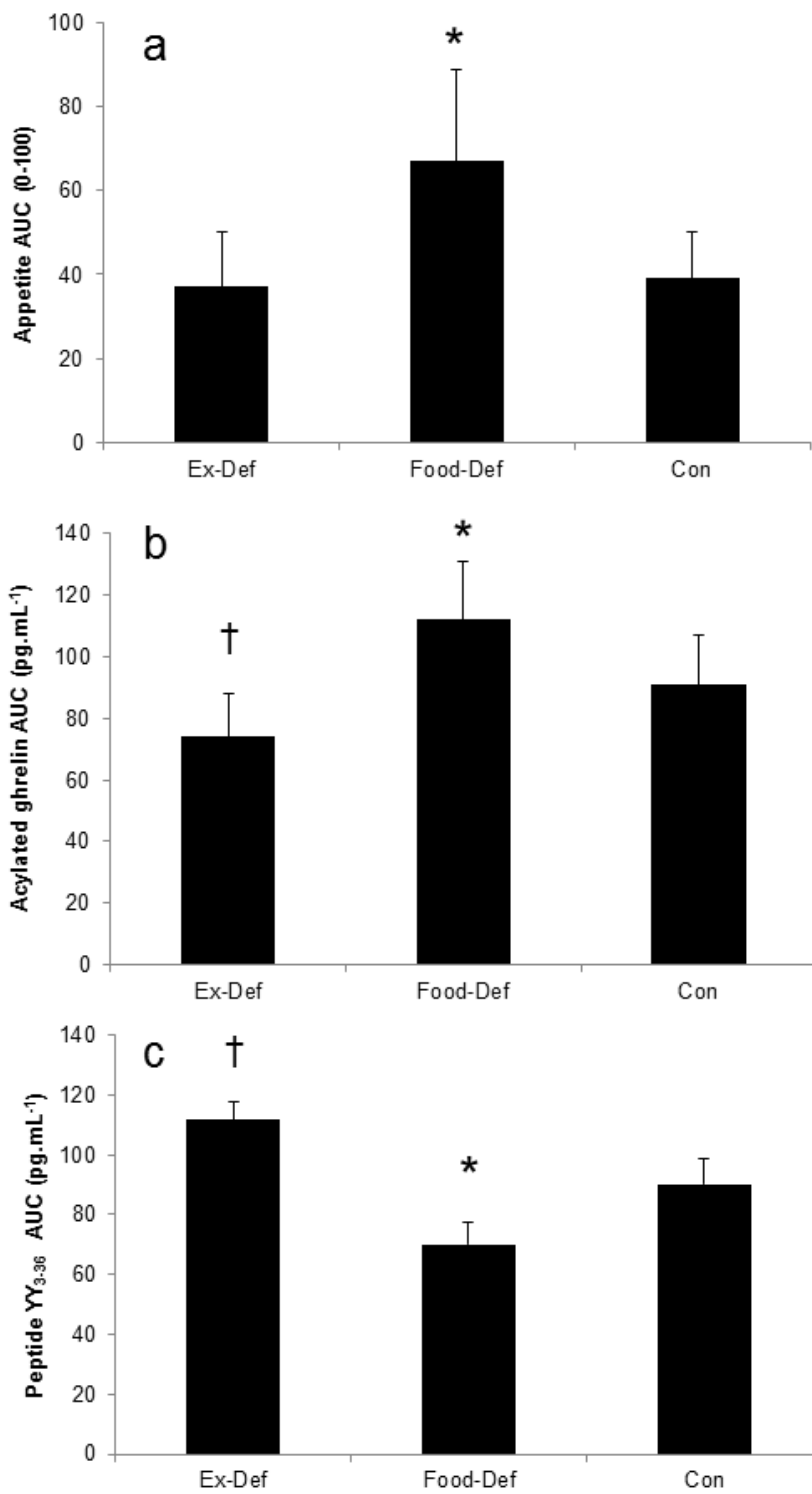
608 **Figure 1.** Time-averaged appetite (a), circulating acylated ghrelin (b) and peptide YY<sub>3-36</sub> (c)  
609 AUC for each 9 h trial. \*Food-Def significantly different from Ex-Def and control; † Ex-Def  
610 significantly different from Food-Def and control (experiment one – female participants  
611 only). Values are mean (SEM), N = 12 for appetite and 11 for acylated ghrelin and peptide  
612 YY<sub>3-36</sub>.

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

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

624 **Figure 4.** (a) Plasma acylated ghrelin concentrations in Male Con (○), Male Ex (●), Female  
625 Con (▽) and Female Ex (▼) (experiment two – male and female participants). Hatched  
626 shaded rectangles indicate standardised test meal, lightly shaded rectangle indicates exercise,  
627 black rectangle indicates *ad libitum* meal. (b) Time-averaged acylated ghrelin AUC for each  
628 7 h trial. ¶ Females significantly different than males. § Control significantly different than  
629 exercise. Values are mean (SEM). Females N=8; males N=8.

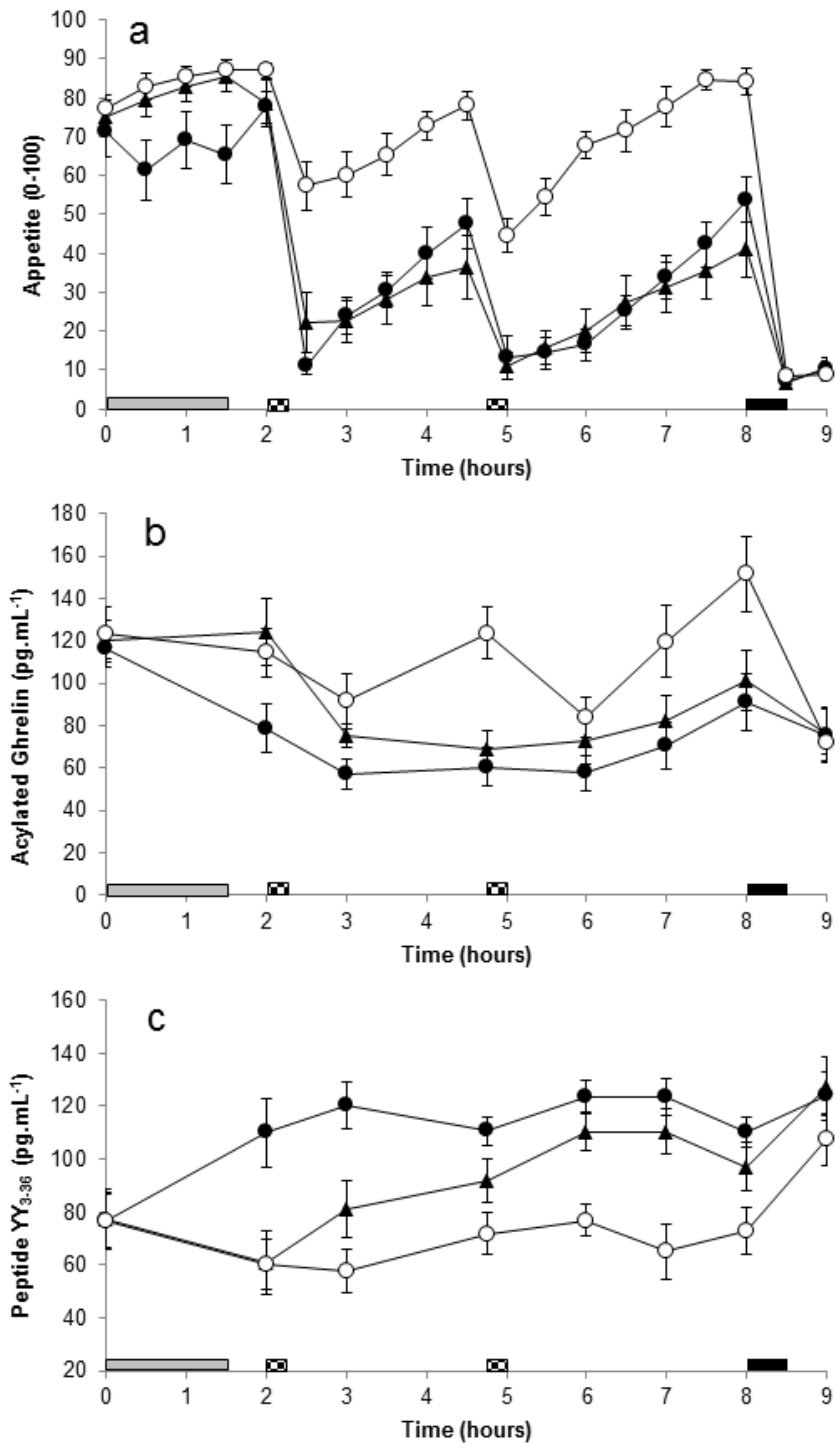
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634 **Figure 2**



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Figure 3

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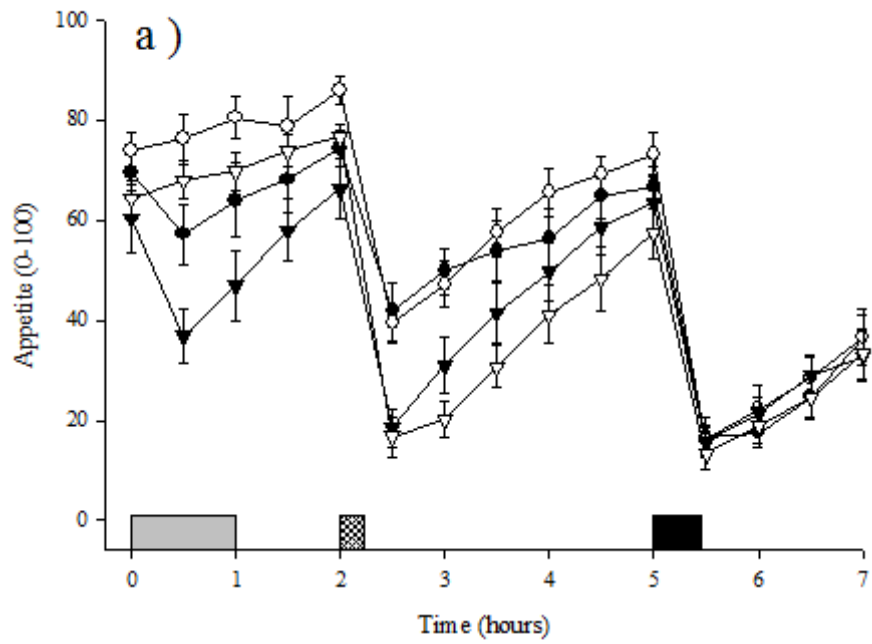
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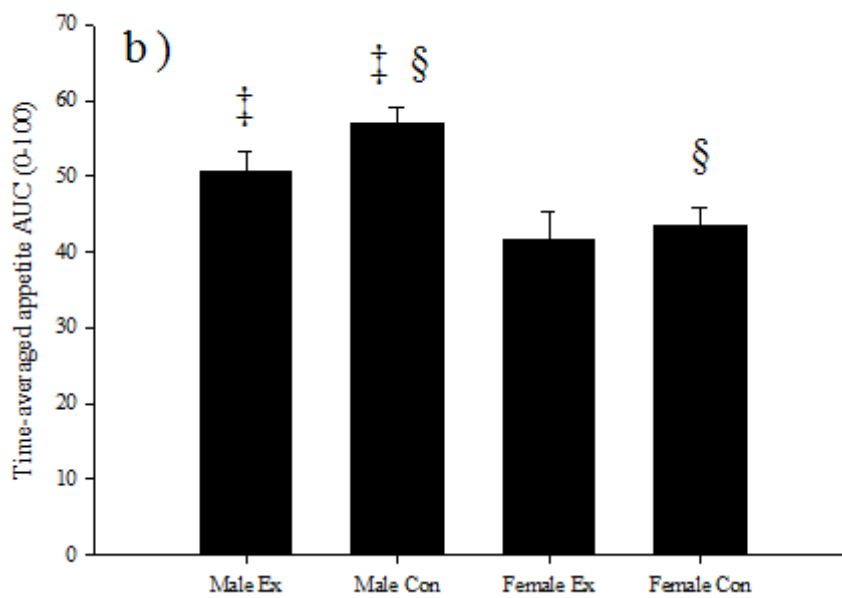
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Figure 4

