



LEEDS
BECKETT
UNIVERSITY

Citation:

Deighton, K and Batterham, RL and Stensel, DJ (2014) Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite*, 81. 52 - 59. ISSN 0195-6663 DOI: <https://doi.org/10.1016/j.appet.2014.06.003>

Link to Leeds Beckett Repository record:

<https://eprints.leedsbeckett.ac.uk/id/eprint/133/>

Document Version:

Article (Accepted Version)

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please [contact us](#) and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on openaccess@leedsbeckett.ac.uk and we will investigate on a case-by-case basis.

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

3 Kevin Deighton^{a, b}, Rachel L Batterham^c and David J Stensel^a

4 ^aSchool of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, LE11
5 3TU, United Kingdom.

6 ^bSchool of Sport, Leeds Metropolitan University, Leeds, LS6 3QS, United Kingdom.

7 ^cCentre for Obesity Research, Department of Medicine, University College London, London,
8 WC1E 6JJ, United Kingdom.

9

10 **Correspondence**

11 Kevin Deighton

12 School of Sport,

13 Leeds Metropolitan University,

14 Leeds

15 LS6 3QS

16 United Kingdom

17 Phone: +44 (0)113 81 25191

18 E-mail: K.Deighton@leedsmet.ac.uk

19

20 **Abstract**

21 Weight loss is the result of a sustained negative energy balance, which is typically achieved by
22 decreasing food intake and/or increasing physical activity. Current evidence suggests that acute
23 energy deficits of ~4820kJ elicit contrasting homeostatic responses when induced by exercise and
24 food restriction but the response to government-recommended energy deficits is unknown. Twelve
25 healthy men (mean(SD): age 24(5)years, body mass index 23.8(2.7)kg.m⁻², maximum oxygen
26 uptake 55.4(9.1)mL.kg⁻¹.min⁻¹) completed three 8h trials (control (Con), exercise-induced energy
27 deficit (Ex-Def) and food restriction (Food-Def)) separated by 1 week. Thirty minutes of cycling at
28 64.5(3.2)% of maximum oxygen uptake was performed in Ex-Def from 0-0.5h, which induced an
29 energy deficit of 1469(256)kJ. An equivalent energy deficit was induced in Food-Def
30 (1478(275)kJ) by reducing the energy content of standardised test meals at 1h and 4h. Appetite
31 ratings, acylated ghrelin and peptide YY₃₋₃₆ concentrations were measured throughout each trial. An
32 *ad libitum* meal was provided at 7h. Appetite was higher in Food-Def than Ex-Def from 4-8h
33 (P=0.033) and tended to be higher across the entire 8h trial (P=0.059). However, energy intake at
34 the *ad libitum* meal did not differ between trials (P = 0.634; Con 4376 (1634); Food-Def 4481
35 (1846); Ex-Def 4217 (1850) kJ). Acylated ghrelin was not related to changes in appetite but plasma
36 PYY₃₋₃₆ concentrations were higher in Ex-Def than Food-Def (P<0.05) and negatively correlated
37 with changes in appetite across the entire 8h trial (P=0.037). An energy deficit of ~1475kJ
38 stimulated compensatory increases in appetite when induced via calorie restriction but not when
39 achieved by an acute bout of exercise. Appetite responses were associated with changes in plasma
40 PYY₃₋₃₆ but not acylated ghrelin concentrations and did not influence subsequent energy intake.

41

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

43 compensation; energy intake

44 **Introduction**

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

52 For weight loss to occur, a sustained negative energy balance is required and is typically achieved
53 by decreasing energy intake (i.e. dieting) and/or increasing energy expenditure (i.e. exercising).
54 Although both interventions induce a negative energy balance, current research suggests that
55 exercise and caloric restriction elicit contrasting homeostatic responses. In this regard, acute caloric
56 restriction appears to stimulate rapid compensatory increases in appetite and energy intake that do
57 not occur in response to equivalent energy deficits induced by exercise (Hubert et al., 1998; King et
58 al., 2011a). Furthermore, King et al. (2011a) reported immediate decreases in circulating
59 concentrations of the anorectic gut hormone PYY₃₋₃₆ and increases in the orexigenic gut hormone
60 acylated ghrelin in response to food restriction but no compensatory changes in response to
61 exercise. Such findings suggest that these appetite-regulating gut hormones have a mediating role in
62 the immediate appetite and energy intake responses to acute energy deficits but this requires further
63 investigation.

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

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

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

84 The purpose of this study was to investigate the appetite, acylated ghrelin, PYY₃₋₃₆ and energy
85 intake responses to a 30 min bout of moderate intensity cycling compared with an equivalent energy
86 deficit achieved via caloric restriction. This study also enables further investigation into the
87 sensitivity of the appetite-regulating system and the role of acylated ghrelin and PYY₃₋₃₆ in energy
88 homeostasis via the utilisation of small, yet practical, energy deficits. It was hypothesised that
89 appetite and acylated ghrelin would increase, and that PYY₃₋₃₆ would decrease in response to food
90 restriction but that these variables would remain unaffected by exercise, resulting in a higher energy
91 intake in the food restriction trial.

92 **Methods**

93 *Participants*

94 This study was conducted according to the guidelines laid down in the Declaration of Helsinki and
95 all procedures involving human participants were approved by the Loughborough University Ethics
96 Advisory Committee (reference number: R12-P61). Written informed consent was obtained from all
97 participants. Participants were male, non-smokers, not taking medication, weight stable for at least
98 6 months before the study and were not dieting. The physical characteristics of participants (mean
99 (SD)) were as follows: age 24 (5) years, body mass index (BMI) 23.8 (2.7) kg.m⁻², body mass 75.3
100 (10.3) kg, body fat 14.2 (4.0) %, waist circumference 80.3 (6.6) cm, maximum oxygen uptake (VO₂
101 max) 55.4 (9.1) mL.kg⁻¹.min⁻¹.

102 *Preliminary Trials*

103 Prior to main trials participants visited the laboratory for two preliminary trials. During the first
104 visit, preliminary anthropometric measurements were collected and participants completed a
105 maximal exercise test to determine VO₂ max. Height and body weight were measured and BMI was
106 subsequently calculated. Body fat percentage was estimated via skinfold measurements of the
107 biceps, triceps, sub-scapular and suprailiac sites (Durnin & Womersley, 1974) and waist
108 circumference determined as the narrowest part of the torso between the xiphoid process and the
109 iliac crest. Maximum oxygen uptake was determined using a continuous incremental cycle test to
110 exhaustion as described previously (Deighton et al., 2013a). Acceptability of the food items to be
111 provided during the main trials was assessed by completion of a food preference questionnaire. The
112 questionnaire required participants to rate preselected food items on a scale ranging from 1 (dislike
113 extremely) to 10 (like extremely). Any volunteers that scored ≤5 for any of the pre-selected food
114 items to be presented were excluded from participating in the study.

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

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

123 *Experimental Protocol*

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

132 During each trial, appetite perceptions (hunger, satisfaction, fullness and prospective food
133 consumption) (Flint et al., 2000) were assessed at baseline, 0.25, 0.5 h and every 30 min thereafter
134 using 100 mm visual analogue scales. An overall appetite rating was calculated as the mean value
135 of the four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al.,
136 2000).

137 *Test Meals*

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

140 content of the meal was 72.9 % carbohydrate, 9.5 % protein and 17.6 % fat. A standardised lunch
141 was provided at 4 h (~12pm) and consisted of a tuna and mayonnaise sandwich, salted crisps,
142 chocolate muffin and green apple. The macronutrient content of the meal was 47% carbohydrate,
143 17.6% protein and 35.4% fat.

144 *Energy Deficits*

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

151 The energy content of the test meals was identical in Con and Ex-Def. The breakfast meal provided
152 30 % and the lunch meal 35 % of the estimated daily energy needs of each individual for a
153 sedentary day, which was calculated using the Mifflin-St Jeor equation and a physical activity
154 factor of 1.4 (Mifflin et al., 1990). The mean (SD) energy intake at breakfast and lunch in Con and
155 Ex-Def was 3074 (221) kJ and 3587 (258) kJ. This equated to a breakfast composition of: 171.3
156 (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
157 (12.3) g orange juice. The average lunch composition was as follows: 103.4 (7.4) g bread, 11.4 (0.8)
158 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
159 (8.7) g apple.

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

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

165 *Ad Libitum Meal*

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

177 *Blood Sampling*

178 Upon arrival to the laboratory, participants rested in a semi-supine position and a cannula (Venflon,
179 Becton Dickinson, Helsinborg, Sweden) was inserted into an antecubital vein. Blood samples were
180 collected at baseline, 1, 2.5, 4, 5, 6, 7 and 8 h for the determination of plasma acylated ghrelin and
181 PYY₃₋₃₆ concentrations. To prevent the degradation of acylated ghrelin, blood samples were
182 collected into pre-chilled 4.9 mL monovettes containing a 50 µl solution of potassium phosphate
183 buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH). These
184 monovettes were spun at 1165 x g for 10 min at 4°C. The plasma supernatant was then dispensed
185 into a storage tube and 100 µl of 1M hydrochloric acid was added per millilitre of plasma to

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

188 For the determination of plasma PYY₃₋₃₆ concentrations, blood samples were collected into pre-
189 chilled syringes containing 10 µl DPP-IV inhibitor (Millipore, Watford, UK) per mL of blood.
190 Syringes were then inverted and the blood dispensed into pre-chilled 2 mL EDTA tubes containing
191 500 KIU aprotonin (Nordic Pharma, Reading, UK) per mL of blood. Blood tubes were promptly
192 centrifuged at 1165 × g for 10 min at 4 °C. The plasma supernatant was stored at -20°C for later
193 analysis.

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

198 *Biochemical Analysis*

199 A commercially available enzyme immunoassay was used to determine plasma concentrations of
200 acylated ghrelin (SPI BIO, Montigny le Bretonneux, France). Plasma concentrations of PYY₃₋₃₆
201 were determined using a commercially available radioimmunoassay (Millipore, Watford, UK). To
202 eliminate interassay variation, samples from each participant were analysed in the same run. The
203 within batch coefficient of variation for the assays were 6.8 and 7.2 % for acylated ghrelin and
204 PYY₃₋₃₆, respectively.

205 *Statistical Analysis*

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

209 AUC values for appetite, acylated ghrelin and PYY₃₋₃₆. Where significant main effects of trial were
210 found, post-hoc analysis was performed using Holm-Bonferroni correction for multiple
211 comparisons. In accordance with previous research (Deighton et al., 2013b; Stoeckel et al., 2008)
212 acylated ghrelin and PYY₃₋₃₆ concentrations are presented as delta values in order to minimise the
213 influence of day-to-day biological variations in these hormones. Correction of acylated ghrelin and
214 PYY₃₋₃₆ concentrations for changes in plasma volume did not alter the interpretation of the results;
215 therefore, for simplicity, the unadjusted values are presented. Statistical significance for this study
216 was accepted as $P < 0.05$. Results in text and tables are presented as mean (SD). Graphical
217 representations of results are presented as mean (SEM) to avoid distortion of the graphs. Based on
218 previous data from our laboratory (Deighton et al., 2013a), a sample size of 12 participants was
219 determined as sufficient to detect a 10 % difference in appetite perceptions during the post-exercise
220 period. This calculation was performed using G*power with an alpha value of 5 % and a power of
221 80 % (Faul et al., 2007).

222 **Results**

223 *Exercise responses*

224 Participants completed the 30 min cycle at 186 (38) W. This elicited an oxygen consumption
225 equivalent to 64.5 (3.2) % of VO₂ max and a net energy expenditure of 1469 (256) kJ. The non-
226 protein respiratory exchange ratio was 0.93 (0.04), which reflected a proportional contribution to
227 energy provision of 78 (13) % carbohydrate and 22 (13) % fat. Heart rate and RPE were 156 (16)
228 beats.min⁻¹ and 13 (1), respectively.

229 *Appetite*

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

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

236 *Energy intake*

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

241 One-way ANOVA revealed no between trial differences in the amount of food consumed at the *ad*
242 *libitum* meal ($P = 0.760$; Con 764.6 (295.4); Food-Def 765.9 (307.7); Ex-Def 734.5 (313.4) g).
243 Consequently energy intake did not differ between trials ($P = 0.634$; Con 4376 (1634); Food-Def
244 4481 (1846); Ex-Def 4217 (1850) kJ). This resulted in an energy balance that was 1628 (915) kJ
245 and 1373 (1047) kJ lower in Ex-Def and Food-Def compared with Con (both $P \leq 0.001$).

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

249 *Plasma acylated ghrelin concentrations*

250 Fasting plasma acylated ghrelin concentrations did not differ significantly between trials at baseline
251 (Con 189 (262); Ex-Def 242 (386); Food-Def 268 (427) $\text{pg}\cdot\text{mL}^{-1}$; $P = 0.174$). Delta AUC for
252 acylated ghrelin concentrations tended to be higher in Con than Ex-Def and Food-Def from 0-1 h (P
253 $= 0.081$) but did not differ between trials for any other time period (1-4 h: $P = 0.116$; 4-8 h: $P =$
254 0.217 ; 0-8 h: $P = 0.160$; Figure 2a).

255 Subsequent boxplot analysis of acylated ghrelin AUC values revealed three consistently outlying
256 participants within the data set (Field, 2009). These participants exhibited fasting acylated ghrelin
257 concentrations that were between 6 and 39 standard deviations higher than the mean fasting value
258 of the remaining nine participants on all trials. In accordance with previous research, these three
259 participants were removed from the data set for subsequent analysis (Broom et al., 2007; Hansen et
260 al., 2002; King et al., 2011b). After the removal of these participants from the data, one-way
261 ANOVA revealed significantly lower delta acylated ghrelin concentrations from 0 – 1 h in Ex-Def
262 compared with Con and Food-Def ($P < 0.05$). There was also a tendency for depressed values in
263 Ex-Def compared with Con and Food-Def from 1 – 4 h ($P = 0.069$) and across the entire 8 h trial (P
264 $= 0.075$) (Figure 2b). Removal of the outliers did not affect the interpretation of the appetite or
265 PYY₃₋₃₆ findings. Plasma acylated ghrelin concentrations for one outlying participant are displayed
266 in Figure 2c in order to highlight the variation in acylated ghrelin profiles.

267 *Peptide YY₃₋₃₆ concentrations*

268 Fasting PYY₃₋₃₆ concentrations did not differ significantly between trials at baseline (Con 93.5
269 (40.0); Ex-Def 87.1 (37.9); Food-Def 96.7 (46.0) pg.mL⁻¹; $P = 0.325$). Delta AUC for plasma PYY₃₋
270 ₃₆ concentrations were significantly higher in Ex-Def than Con and Food-Def from 0 – 1 h ($P <$
271 0.01) and in Ex-Def compared with Food-Def from 1 - 4 h and across the entire 8 h trial ($P < 0.05$)
272 (Figure 3; Table 2).

273 *Correlations*

274 Area under the curve values for delta PYY₃₋₃₆ concentrations were negatively correlated with
275 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
276 entire 8 h trial (0 - 8 h; $r = -0.349$; $P = 0.037$). There were no significant correlations between
277 acylated ghrelin and appetite AUCs for any time period. The *ad libitum* energy intake response to
278 exercise and food restriction was not significantly correlated with any of the participant

279 characteristics including age, height, weight, BMI, body fat, waist circumference and VO₂ max (all
280 P > 0.18).

281 **Discussion**

282 The primary finding of this investigation is that an energy deficit of ~1475 kJ stimulated
283 compensatory increases in appetite when induced via food restriction but not when achieved by an
284 acute bout of exercise. These divergent appetite responses were associated with changes in
285 circulating concentrations of PYY₃₋₃₆ but were unrelated to changes in plasma acylated ghrelin and
286 did not influence subsequent energy intake.

287 This study has extended the findings of previous research by demonstrating that appetite
288 perceptions increase in response to subtle reductions in energy intake but do not change in response
289 to an equivalent exercise-induced energy deficit (Hubert et al., 1998; King et al., 2011a). Increases
290 in appetite occurred despite an average decrease in energy intake of only 682 kJ at breakfast and
291 796 kJ at lunch. This highlights the sensitivity of the appetite-regulating system to reductions in
292 food intake and supports previous observations that dieting is often compromised by increases in
293 appetite (Ikeda et al., 2004). Additionally, the observed increase in appetite in response to food
294 restriction across two meals was smaller than that previously reported for a similar energy deficit
295 induced at a single meal (Hubert et al., 1998). This suggests that creating an energy deficit across
296 multiple meals may be more effective for minimising increases in appetite than at a single meal but
297 this requires further investigation. In contrast, appetite was unaltered in response to an equivalent
298 energy deficit induced through 30 min of moderate intensity exercise. This exercise bout represents
299 the current UK government and ACSM guidelines for physical activity (British Heart Foundation,
300 2009; Donnelly et al., 2009) and supports previous findings that an acute bout of continuous
301 moderate intensity exercise does not stimulate compensatory increases in appetite during the
302 subsequent hours (Deighton & Stensel, 2014).

303 In contrast with previous findings, the divergent appetite response to exercise and food restriction
304 was not associated with concordant changes in plasma acylated ghrelin concentrations (King et al.,
305 2011a). Furthermore, the acylated ghrelin profile of the participant displayed in Figure 2c exhibited
306 an increase in response to the lunch meal in all trials despite reporting a simultaneous decrease in
307 appetite. Such disassociation between appetite and ghrelin profiles in a single participant has
308 previously been reported by Cummings et al. (2004), as one out of six participants did not
309 demonstrate an increase in ghrelin prior to spontaneous meal request, despite exhibiting significant
310 increases in appetite and a similar energy intake and meal request response as all other participants.
311 The reasons for the occurrence of outlying participants in the present study are unclear as all
312 outliers displayed an appetite, energy intake and PYY₃₋₃₆ response that was consistent with the
313 remainder of the sample. Furthermore, there was no difference between the outlying and non-
314 outlying participants for any of the measured physiological characteristics. In order to further
315 investigate the mechanisms underlying the disassociation between appetite perceptions and ghrelin
316 concentrations in some participants, it may be beneficial for future experiments to also measure
317 circulating insulin levels as an inverse relationship between ghrelin and insulin concentrations has
318 been previously reported (Cummings et al., 2004; Flanagan et al., 2003).

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

326 The findings of the present study contribute to the current debate about the importance of
327 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 PYY₃₋₃₆ 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 PYY₃₋₃₆ 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 PYY₃₋₃₆ after
346 exercise. Furthermore, although not statistically significant, the increase in PYY₃₋₃₆ concentrations
347 in response to the lunch meal appeared to be reduced during the food restriction trial. Considering
348 the prominent role of PYY₃₋₃₆ as a mediator of satiety (Batterham et al., 2007), it seems plausible
349 that the contrasting changes in PYY₃₋₃₆ 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

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

358 Surprisingly, despite a significant increase in appetite in response to caloric restriction, energy
359 intake at the *ad libitum* meal did not differ between trials. This contrasts with previous
360 investigations that have demonstrated an increase in energy intake in response to food restriction
361 compared with an equivalent energy deficit induced via exercise (Hubert et al., 1998; King et al.,
362 2011a). However, this is likely to reflect the smaller changes in appetite observed in the present
363 study due to the modest energy deficits employed. Such a disassociation between appetite and
364 energy intake has been commonly reported within the scientific literature in response to modest
365 experimental manipulations and is thought to represent an accruing degree of motivation prior to the
366 initiation of a behavioural response (Stubbs et al., 2000). The uncoupling between appetite
367 perceptions and energy intake may also have been influenced by the sedentary activities of
368 participants between the lunch and *ad libitum* meal. In this regard, research has demonstrated that
369 sedentary activities can stimulate hedonic feeding (Chaput et al., 2011), which is likely to have
370 occurred in the present study considering the large *ad libitum* energy intakes despite appetite scores
371 immediately prior to the meal being rated as ~ 60 out of 100. Such high energy intakes may have
372 reduced the sensitivity of the meal to detect changes in energy intakes as a result of the exercise and
373 caloric restriction interventions. It seems reasonable to speculate that continued food restriction
374 would elicit increases in energy intake over a longer monitoring period but this requires further
375 investigation.

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

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

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

405 similar appetite and energy intake responses in lean and obese participants (Ueda et al., 2009),
406 further investigations in overweight and obese populations are needed because this is where weight-
407 management strategies have the most clinical relevance. Additionally, due to the time-constraints of
408 the present study, ad libitum feeding occurred ~3 h after the standardised lunch meal when appetite
409 remained relatively low. This does not represent an ecologically valid scenario and increases the
410 likelihood that food intake during this meal was driven by hedonic rather than homeostatic stimuli.
411 Additionally, the use of a single food item to assess *ad libitum* energy intake prevented any
412 investigation into the effects of the interventions on food choice. However, the use of a single food
413 item allowed a more consistent evaluation of energy intake as the macronutrient content of the meal
414 was fixed. Finally, the mechanistic investigation of this study was limited to the measurement of
415 acylated ghrelin and PYY₃₋₃₆. Future studies may aim to assess changes in additional
416 gastrointestinal hormones including glucagon-like-peptide-1 (GLP-1), pancreatic polypeptide and
417 oxyntomodulin. The measurement of GLP-1 in combination with PYY₃₋₃₆ may be particularly
418 prudent as these hormones have been found to have an additive effect on satiety (De Silva et al.,
419 2011).

420 In conclusion, food restriction of ~1478 kJ across two meals stimulated compensatory increases in
421 appetite that did not occur in response to a similar energy deficit induced by 30 min of moderate
422 intensity exercise. Although the mechanisms underlying such a contrasting response are unclear, it
423 does not appear to be influenced by changes in plasma acylated ghrelin concentrations.
424 Alternatively, changes in PYY₃₋₃₆ were negatively correlated with changes in appetite, which
425 supports the anorexigenic nature of this peptide. Future studies should be conducted to elucidate
426 whether PYY₃₋₃₆ concentrations also increase in response to exercise in obese participants and if this
427 improves the satiety response to a standardised meal.

428 **Acknowledgements**

429 The authors thank Jessica Douglas and Harriet Pryke for their help with the data collection, Jenny
430 Jones and Sean Manning for help with PYY₃₋₃₆ assays and all of the volunteers for their
431 participation in this study. This project received no external funding. The authors declare no conflict
432 of interest.

433 **References**

- 434 Batterham, R. L., Ffytche, D. H., Rosenthal, J. M., Zelaya, F. O., Barker, G. J., Withers, D. J., &
435 Williams, S. C. R. (2007). PYY modulation of cortical and hypothalamic brain areas predicts
436 feeding behaviour in humans. *Nature*, *450*, 106–9.
- 437 Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., le Roux, C.
438 W., et al. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight
439 regulation. *Cell Metab*, *4*, 223–33.
- 440 Borg, G. A. (1973). Perceived exertion: a note on “history” and methods. *Med Sci Sports*, *5*, 90–3.
- 441 Bray, G. A. (2004). Medical consequences of obesity. *J Clin Endocrinol Metab*, *89*, 2583–9.
- 442 British Heart Foundation (2009). Physical Activity Guidelines in the UK: Review &
443 Recommendations. Available at:
444 [https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213743/dh_128255.p](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213743/dh_128255.pdf)
445 [df](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213743/dh_128255.pdf).
- 446 Broom, D. R., Stensel, D. J., Bishop, N. C., Burns, S. F., & Miyashita, M. (2007). Exercise-induced
447 suppression of acylated ghrelin in humans. *J Appl Physiol*, *102*, 2165–71.
- 448 Chaput, J.P., Klingenberg, L., Astrup, A., & Sjodin, A.M. (2011). Modern sedentary activities
449 promote overconsumption of food in our current obesogenic environment. *Obes Rev*, *12*, e12-e20.
- 450 Cheng, M. H.-Y., Bushnell, D., Cannon, D. T., & Kern, M. (2009). Appetite regulation via exercise
451 prior or subsequent to high-fat meal consumption. *Appetite*, *52*, 193–8.
- 452 Cummings, D. E., Frayo, R. S., Marmonier, C., Aubert, R., & Chapelot, D. (2004). Plasma ghrelin
453 levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues.
454 *Am J Physiol Endocrinol Metab*, *287*, E297–304.

455 Curioni, C. C., & Lourenço, P. M. (2005). Long-term weight loss after diet and exercise: a
456 systematic review. *Int J Obes*, 29, 1168–74.

457 De Silva, A., Salem, V., Long, C.J., Makwana, A., Newbould, R.D., Rabiner, E.A., Ghatei, M.A., et
458 al. (2011). The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate
459 brain activity in appetite centres in humans. *Cell Metab*, 14, 700-6.

460 Deighton, K., Barry, R., Connon, C. E., & Stensel, D. J. (2013a). Appetite, gut hormone and energy
461 intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl*
462 *Physiol*, 113, 1147–56.

463 Deighton, K., Karra, E., Batterham, R. L., & Stensel, D. J. (2013b). Appetite, energy intake, and
464 PYY3-36 responses to energy-matched continuous exercise and submaximal high-intensity
465 exercise. *Appl Physiol Nutr Metab*, 38, 947–52.

466 Deighton, K., & Stensel, D.J. (2014). Creating an acute energy deficit without stimulating
467 compensatory increases in appetite: is there an optimal exercise protocol? *Proc Nutr Soc*, 73, 352-8.

468 Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma,
469 and red cells in dehydration. *J Appl Physiol*, 37, 247–8.

470 Donnelly, J. E., Blair, S. N., Jakicic, J. M., Manore, M. M., Rankin, J. W., & Smith, B. K. (2009).
471 American College of Sports Medicine Position Stand. Appropriate physical activity intervention
472 strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*, 41,
473 459–71.

474 Durnin, J. V., & Womersley, J. (1974). Body fat assessed from total body density and its estimation
475 from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J*
476 *Nutr*, 32, 77–97.

477 Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: a flexible statistical power
478 analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*, 39, 175–
479 91.

480 Field, A. (2009). *Discovering Statistics Using SPSS* (Third Edition). Sage, London, UK.

481 Flanagan, D.E., Evans, M.L., Monsod, T.P., Rife, F., Heptulla, R.A., Tamborlane, W.V., &
482 Sherwin, R.S. (2003). The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol*
483 *Metab*, 284, E313-6.

484 Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of
485 visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes*
486 *Relat Metab Disord*, 24, 38–48.

487 Franz, M. J., VanWormer, J. J., Crain, A. L., Boucher, J. L., Histon, T., Caplan, W., Bowman, J. D.,
488 et al. (2007). Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical
489 trials with a minimum 1-year follow-up. *J Am Diet Assoc*, 107, 1755–67.

490 Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl*
491 *Physiol*, 55, 628–34.

492 Hansen, T. K., Dall, R., Hosoda, H., Kojima, M., Kangawa, K., Christiansen, J. S., & Jørgensen, J.
493 O. L. (2002). Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol*,
494 56, 203–6.

495 Hosoda, H., Doi, K., Nagaya, N., Okumura, H., Nakagawa, E., Enomoto, M., Ono, F., et al. (2004).
496 Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of
497 ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clin Chem*, 50, 1077–80.

498 Hubert, P., King, N. A., & Blundell, J. E. (1998). Uncoupling the effects of energy expenditure and
499 energy intake: appetite response to short-term energy deficit induced by meal omission and physical
500 activity. *Appetite*, *31*, 9–19.

501 Ikeda, J. P., Lyons, P., Schwartzman, F., & Mitchell, R. A. (2004). Self-reported dieting
502 experiences of women with body mass indexes of 30 or more. *J Am Diet Assoc*, *104*, 972–4.

503 Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., Ando, T., et al. (2013).
504 Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite*, *66*,
505 26–33.

506 King, J. A., Wasse, L. K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R. L., & Stensel, D. J.
507 (2011a). Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to
508 equivalent energy deficits created by exercise and food restriction. *J Clin Endocrinol Metab*, *96*,
509 1114–21.

510 King, J. A., Wasse, L. K., & Stensel, D. J. (2011b). The acute effects of swimming on appetite, food
511 intake, and plasma acylated ghrelin. *J Obes* 351628.

512 King, N A, Hopkins, M., Caudwell, P., Stubbs, R. J., & Blundell, J. E. (2008). Individual variability
513 following 12 weeks of supervised exercise: identification and characterization of compensation for
514 exercise-induced weight loss. *Int J Obes*, *32*, 177–84.

515 King, N. A., Caudwell, P., Hopkins, M., Byrne, N. M., Colley, R., Hills, A. P., Stubbs, J. R., et al.
516 (2007). Metabolic and behavioral compensatory responses to exercise interventions: barriers to
517 weight loss. *Obesity*, *15*, 1373–83.

518 Korner, J., Bessler, M., Cirilo, L. J., Conwell, I. M., Daud, A., Restuccia, N. L., & Wardlaw, S. L.
519 (2005). Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of
520 plasma ghrelin, peptide YY, and insulin. *J Clin Endocrinol Metab*, *90*, 359–65.

521 le Roux, C. W., Batterham, R. L., Aylwin, S. J. B., Patterson, M., Borg, C. M., Wynne, K. J., Kent,
522 A., et al. (2006). Attenuated peptide YY release in obese subjects is associated with reduced satiety.
523 *Endocrinology*, *147*, 3–8.

524 Lippl, F., Erdmann, J., Steiger, A., Lichter, N., Czogalla-Peter, C., Bidlingmaier, M., Tholl, S., et al.
525 (2012). Low-dose ghrelin infusion--evidence against a hormonal role in food intake. *Regul Pept*,
526 *174*, 26–31.

527 Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A
528 new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*, *51*,
529 241–7.

530 Murphy, K. G., & Bloom, S. R. (2006). Gut hormones and the regulation of energy homeostasis.
531 *Nature*, *444*, 854–9.

532 NHS Choices (2011). Why most of us should eat fewer calories. Available from:
533 <http://www.nhs.uk/Livewell/Goodfood/Pages/eat-less.aspx>.

534 Parr, E.B., Coffey, V.G., & Hawley, J.A. (2013). 'Sarcobesity': a metabolic conundrum. *Maturitas*,
535 *74*, 109-13.

536 Stewart, W. K., & Fleming, L. W. (1973). Features of a successful therapeutic fast of 382 days'
537 duration. *Postgrad Med J*, *49*, 203–9.

538 Stock, S., Lechner, P., Wong, A. C. K., Ghatei, M. A., Kieffer, T. J., Bloom, S. R., & Chanoine, J.-
539 P. (2005). Ghrelin, peptide YY, glucose-dependent insulintropic polypeptide, and hunger
540 responses to a mixed meal in anorexic, obese, and control female adolescents. *J Clin Endocrinol*
541 *Metab*, *90*, 2161–8.

542 Stoeckel, L. E., Weller, R. E., Giddings, M., & Cox, J. E. (2008). Peptide YY levels are associated
543 with appetite suppression in response to long-chain fatty acids. *Physiol Behav*, *93*, 289–95.

544 Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., Elia, M., Stratton, R., et al.
545 (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of
546 their reliability and validity with an evaluation of new hand-held computerized systems for
547 temporal tracking of appetite ratings. *Br J Nutr*, *84*, 405–15.

548 Sumithran, P., Prendergast, L. A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A., & Proietto,
549 J. (2011). Long-term persistence of hormonal adaptations to weight loss. *New Engl J Med*, *365*,
550 1597–604.

551 Sun, Y., Butte, N. F., Garcia, J. M., & Smith, R. G. (2008). Characterization of adult ghrelin and
552 ghrelin receptor knockout mice under positive and negative energy balance. *Endocrinology*, *149*,
553 843–50.

554 Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., & Fujimoto, S. (2009). Changes in gut
555 hormone levels and negative energy balance during aerobic exercise in obese young males. *J*
556 *Endocrinol*, *201*, 151-9.

557 Wasse, L. K., Sunderland, C., King, J. A., Miyashita, M., & Stensel, D. J. (2013). The influence of
558 vigorous running and cycling exercise on hunger perceptions and plasma acylated ghrelin
559 concentrations in lean young men. *Appl Physiol Nutr Metab*, *38*, 1–6.

560 Weigle, D. S., Cummings, D. E., Newby, P. D., Breen, P. A., Frayo, R. S., Matthys, C. C.,
561 Callahan, H. S., et al. (2003). Roles of leptin and ghrelin in the loss of body weight caused by a low
562 fat, high carbohydrate diet. *J Clin Endocrinol Metab*, *88*, 1577–86.

563 World Health Organisation (2009). Global health risks. Available at:
564 http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf.

565 Zhao, T.-J., Liang, G., Li, R. L., Xie, X., Sleeman, M. W., Murphy, A. J., Valenzuela, D. M., et al.
566 (2010). Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of
567 calorie-restricted mice. *Proc Natl Acad Sci USA*, *107*, 7467–72.

568

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

	Preprandial	Morning	Afternoon	Total trial
	(0 - 1 h)	(1- 4 h)	(4 - 8 h)	(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 PYY₃₋₃₆ concentrations in the Control, Ex-Def, and Food-Def trials.

	Preprandial	Intertest meal	Posttest meals	Total Trial
	(0 – 1 h)	(1 – 4 h)	(4 – 8 h)	(0 – 8 h)
Delta PYY₃₋₃₆				
(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 ^{*†}	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 PYY₃₋₃₆ 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

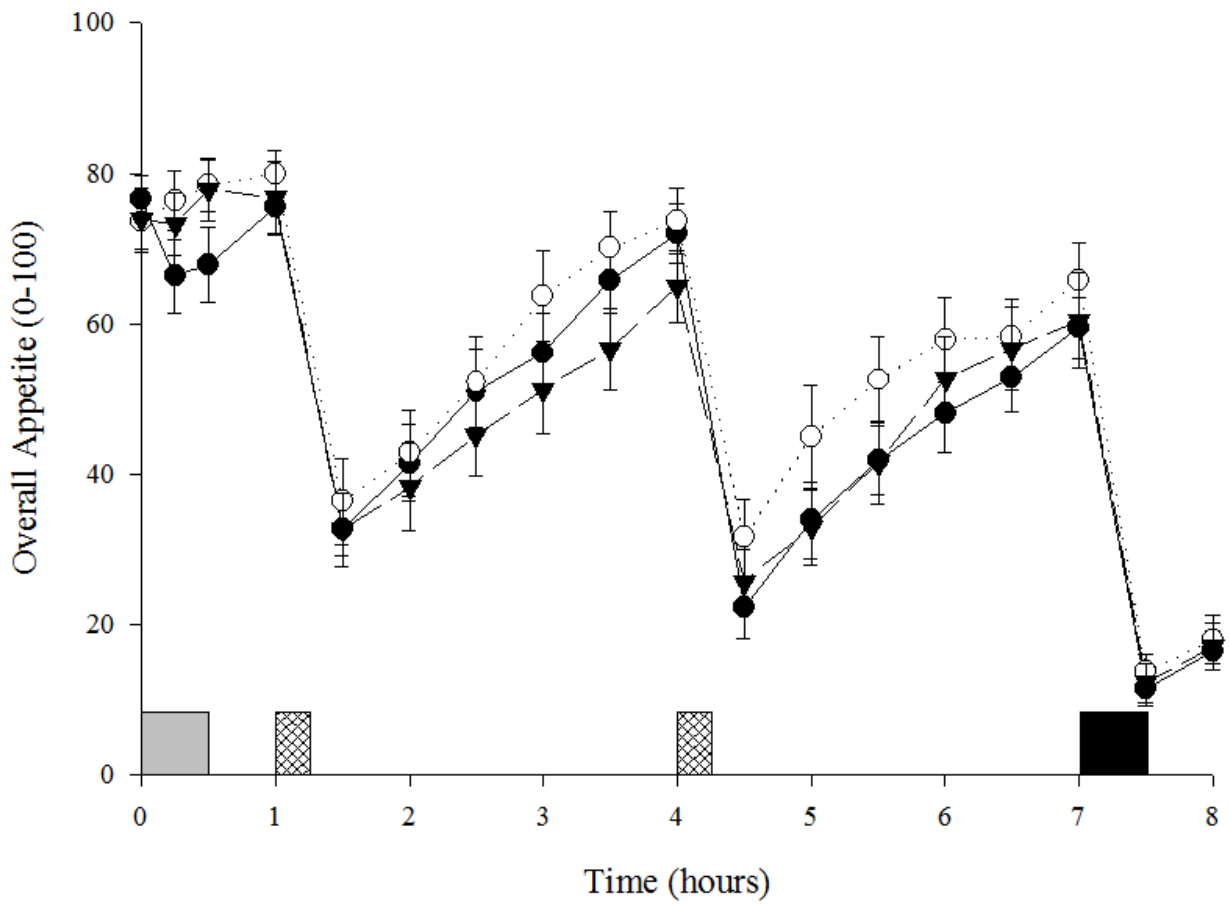


Figure 2

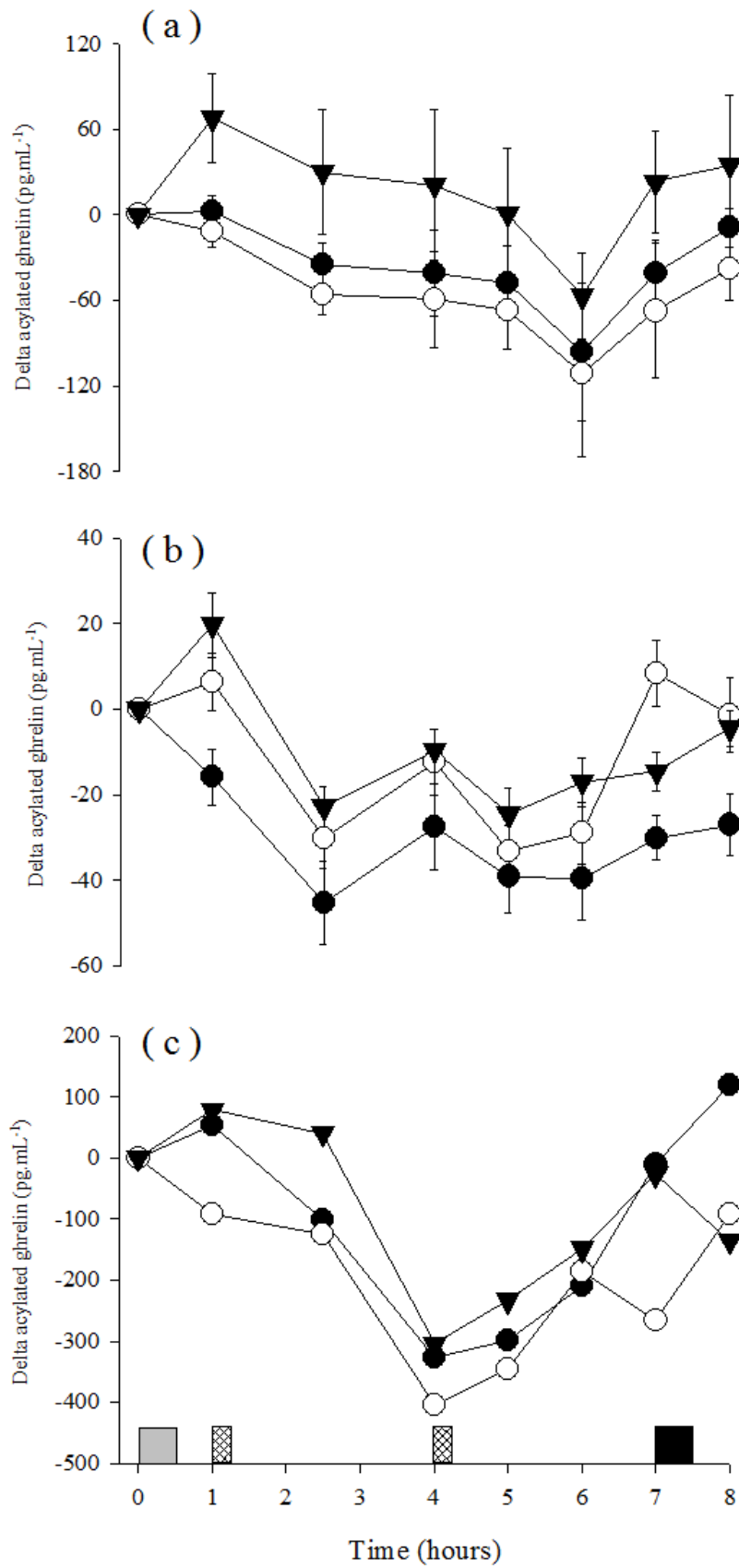


Figure 3

