Appetite regulatory hormone responses on the day following a prolonged bout of moderate-intensity exercise

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Running Head: Latent Appetite Regulatory Responses Following Exercise

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Abstract

Key words: Exercise, Appetite Regulation, Gut Peptides

Abstract

Exercise increases energy expenditure however acutely this does not cause compensatory changes in appetite or food intake. This unresponsiveness contrasts the rapid counter regulatory changes seen after food restriction. The present investigation examined whether corrective changes in appetite regulatory parameters occur after a time delay, namely, on the day after a single bout of exercise. Nine healthy males completed two, two-day trials (exercise & control) in a random order. On the exercise trial participants completed 90 min of moderate intensity treadmill running on day one (10:30 – 12:00 h). On day two appetite regulatory hormones and subjective appetite perceptions were assessed frequently in response to two test meals provided at 08:00 and 12:00 h. Identical procedures occurred in the control trial except no exercise was performed on day one. Circulating levels of leptin were reduced on the day after exercise (AUC 5841 ± 3335 vs. 7266 ± 3949 $ng^{-1}{\cdot}mL^{-1}{\cdot}7$ h, P = 0.012). Conversely, no compensatory changes were seen for circulating acylated ghrelin, total PYY, insulin or appetite perceptions. Unexpectedly, levels of acylated ghrelin were reduced on the exercise trial following the second test meal on day two (AUC 279 ± 136 vs. 326 ± 136 $pg^{-1}{\cdot}mL^{-1}{\cdot}3$ h, P = 0.021). These findings indicate that short-term energy deficits induced by exercise initially prompt a compensatory response by chronic but not acute hormonal regulators of appetite and energy balance. Within this 24 h time-frame however there is no conscious recognition of the perturbation to energy balance.

Introduction

The relationship between exercise and appetite regulation has important implications regarding the role of exercise in weight management (33). In recent years, advancements in scientific understanding regarding the psycho-biological regulation of appetite and food intake have ignited research interest around the interaction between exercise, appetite regulation and energy balance (47). Within this sphere, one particular issue that has received significant attention is the impact of exercise on hormonal mediators of appetite which are central components of the body's homeostatic system governing energy balance and weight control (28, 49).

The body's appetite regulatory system includes several peptides of gastro-intestinal, pancreatic and adipose tissue origin, which communicate acute nutrient status and chronic energy availability to the central nervous system (28). Leptin and insulin act as chronic mediators of energy balance, with circulating concentrations being present in proportion to stored energy within adipose tissue (40). Additionally, on a meal to meal basis, food intake is regulated by a selection of gastrointestinal peptides, most notably acylated ghrelin, peptide-YY (PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK) and oxyntomodulin (44). Ghrelin is secreted from the stomach and remains unique as the only circulating appetite stimulating hormone. Circulating concentrations of ghrelin rise and fall before and after meals, data which implicates ghrelin as meal initiating signal (12, 13). Conversely, each of the other short-acting peptides has an inhibitory effect on appetite. Most prominent is PYY which is secreted chiefly from the distal intestine and colon in direct proportion to the energy content of an ingested meal (1, 37). Within key appetite regulatory brain centres these afferent signals are integrated and the summed response initiated

52 which impacts directly up on appetite and eating, as well as thermogenesis and
53 substrate metabolism (43).
54
55 The last 10 years has seen an explosion of research exploring the links between
56 appetite and appetite regulatory hormones in the context of exercise (47, 49).
57 Research has demonstrated that single bouts of exercise have a marked impact on
58 the circulating levels of appetite regulatory hormones with changes occurring rapidly
59 after the initiation of exercise. Notably however, these alterations appear to be
60 transient. For example, circulating levels of acylated ghrelin are distinctly suppressed
61 during exercise of moderate intensity or higher (10, 29, 31). This perturbation
62 however is absent within 30 min after exercise. Similarly, circulating concentrations
63 of PYY increase during moderate to high intensity exercise however customary
64 levels are re-established shortly thereafter (9, 51). Each of these responses is
65 consistent with an appetite inhibitory profile which may in part contribute to a well
66 characterised inhibition of appetite at moderate-high exercise intensities, a
67 phenomena which has been termed ‘exercise induced anorexia’ (32).
68
69 Studies have shown that acute energy deficits induced by food restriction lead to
70 rapid and quite striking compensatory alterations to appetite and appetite regulatory
71 hormones (27, 31). Intuitively, it may be expected that energy deficits induced by
72 exercise would lead to similar changes in appetite regulatory parameters in an effort
73 to maintain energy balance. Paradoxically, several studies have failed to observe
74 any compensatory changes in circulating appetite hormones (acylated ghrelin or
75 PYY) even after bouts of exercise associated with high levels of energy expenditure
76 and over several hours of observation afterwards (29, 31, 51). It remains possible

that compensatory appetite regulatory changes may occur over a greater period of time than what has previously been examined i.e. beyond the day that exercise is completed on.

To test this hypothesis the current study assessed circulating levels of key appetite regulatory hormones (acylated ghrelin, total PYY, leptin & insulin) and subjective appetite perceptions on the day after a single bout of exercise with a large associated energy deficit. We hypothesised that meal stimulated acylated ghrelin (suppression) and PYY (elevation) responses would be attenuated on the day after exercise whilst circulating levels of leptin would be reduced. Furthermore, we thought that these changes would be associated with higher subjective ratings of appetite.

Materials & Methods

Participants

After receiving local ethical advisory committee approval nine young, healthy male volunteers (age 22 ± 1.2 y; BMI 22.6 ± 1.8 kg·m^2; waist circumference 74.4 ± 1.8 cm; estimated basal metabolic rate 7247 ± 405 kJ; $\dot{V}O_2$max 60.6 ± 7.6 mL·kg·min^{-1}) gave their written informed consent to participate. Participants were weight stable (< 2 kg change in body mass in the last three months), non-smokers, free of cardio-metabolic disease, had a BMI within the healthy range (18.5 – 24.9 kg·m^2) and were not taking any medications or supplements. Participants were recreationally active i.e. typically games players, but were not accustomed to undertaking endurance exercise regularly.

Pre-assessment and Study Familiarisation

Before main trials, participants attended the laboratory where they were familiarised with the study procedures and underwent necessary pre-assessments. Participants completed questionnaires assessing health status and physical activity habits after which measurements of height, weight and waist circumference were taken. Participants then completed two treadmill running tests; 1) a progressive 16 min submaximal test to determine the relationship between treadmill running speed and oxygen consumption; 2) a maximum oxygen uptake test ($\dot{V}O_2$max). These tests have been described in depth previously (10).

Main Experimental Trials

In subsequent weeks participants completed two main experimental trials (exercise and control) separated by a washout period of at least seven days. Each main trial spanned across two days and was preceded by a 48 h lead-in phase where diet and physical activity (absence of) were standardised. Within this standardisation phase dietary intake was controlled by the participants i.e. on each participant's first trial they ate *ad libitum* however participants recorded what they ate and replicated it exactly in the lead up to their second main trial. Adherence to this procedure was confirmed verbally by the study experimenters before main trials. Each main trial was composed of an intervention phase (day one) and a data collection phase (day two). This design permitted the assessment of appetite regulatory responses on the day after exercise. The order of main trials was randomised with five participants completing the control trial first and four completing the exercise trial first. Figure 1 provides a schematic illustration of the main trial protocol.

Main trials began on the morning of day one and ended at approximately 15:10 on day two. During this period participants were required to attend the laboratory between 10:00-13:30 on day one and 07:30-15:10 on day two. In the time away from the laboratory participants were instructed to remain completely inactive and this was checked repeatedly by the study experimenters via telephone. During the study participants travelled to and from the laboratory via motorised transport unless they lived within 400 meters in which case they were permitted to walk. During main trials participants were provided with all of their food which was consumed at set times that were standardised across trials. Water was permitted *ad libitum* on day one however to avoid any impact on appetite and/or gastric function during the data collection phase of trials water consumption was standardised on day two.

On day one of the exercise trial participants consumed their standardised breakfast at home at 07:30. At 10:00 participants arrived at the laboratory ahead of their treadmill run (10:30-12:00). Herein, participants ran on a motorised treadmill (Technogym Excite Med, Cesena, Italy) for 90 min at a speed predicted to elicit 70% of their maximum oxygen uptake. At 15 min intervals oxygen uptake was assessed via expired air collections into a Douglas Bag and the speed of the treadmill was adjusted if necessary to maintain the desired exercise intensity. Ratings of perceived exertion were also assessed using the Borg scale (7). Following the run participants rested in the laboratory until lunch (13:00). After lunch participants went home where they remained (inactive) until returning to the laboratory the following morning. At 18:00 participants consumed their standardised evening meal which was followed by their evening snack at 20:00.

Participants arrived at the laboratory on the morning of day two at 07:15. A cannula was then inserted into an antecubital vein after which participants rested for 30 min. At 08:00 the data collection phase of the trial began whereby baseline blood samples were collected and appetite scales completed. A test meal was then consumed over 10 min. On the final bite a clock was started which ran continuously for seven hours. At 4h a second test meal was consumed. Across this period blood samples were collected for the assessment of appetite regulatory hormones at 0.5, 1, 1.5, 2, 3, 4, 4.5, 5, 5.5, 6 & 7h. Subjective appetite perceptions (hunger, fullness, satisfaction & prospective consumption) were assessed at 30 min intervals throughout using visual analogue scales (18). Main trials ended after the final blood sample/appetite scale at 7 h, at which point the cannula was removed and participants left the laboratory.

Food Provision & Test Meals

On day one of main trials participants received all of their food pre-packaged from the study team with the food provided being identical in the exercise and control trial. The amount of food (energy) each participant received was calculated as 1.4x their estimated basal metabolic rate (42). This is an amount of food deemed sufficient to meet the needs of an individual on an inactive day. On day one breakfast consisted of white bread and chocolate spread (carbohydrate 64%, fat 25%, protein 11% - 20% of daily energy provision). Lunch and dinner was a balanced meal consisting of a tuna and mayonnaise sandwich, salted crisps, chocolate muffin and green apple (carbohydrate 48%, fat 33%, protein 19% - each meal 35% of daily energy provision). Finally, particpants received a chocolate biscuit for the evening snack (carbohydrate 52%, fat 46%, protein 2% - 10% of daily energy provision).

On day two of trials participants received two (baseline and 4 h) balanced (48% carbohydrate, 19% protein, 33% fat, 2565 kJ energy) test meals that were identical within and between trials. Each participant received the exact same meal i.e. the meal was not normalised to participants' daily energy requirements. Each test meal consisted of white bread (109g), cheddar cheese (48g), malt loaf (30g) semi-skimmed milk (100mL) and strawberry milkshake powder (7.5g). Each meal was consumed within 10 min. To keep hydrated participants drank 250 mL of water one hour after each test meal (1 h and 5 h).

Blood Biochemistry

During day two of main trials venous blood samples were collected via a 21G cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) that was kept patent throughout by flushing with isotonic saline (0.9% w/v sodium chloride). Samples were collected into ice-cooled EDTA monovettes for the determination of plasma leptin, insulin and acylated ghrelin. To preserve the integrity of the acylated ghrelin sample, monovettes for this peptide were pre-treated with a serine protease inhibitor as described previously (10). Samples for total PYY were collected into ice-cooled syringes containing 10µL/mL di-peptidyl peptidase-4 inhibitor (Millipore, Watford, UK) and after mixing were immediately dispensed into EDTA tubes containing aprotinin (Nordic Pharma Ltd, Reading, UK) (500 KIU/mL). Plasma was obtained after spinning whole blood samples at 1600 *g* for 10 min in a refrigerated centrifuge (4^{o}C) and was stored at -80^{o}C until analysis. At baseline and 4 h measurements of haematocrit and haemoglobin were taken to estimate changes in plasma volume using the method described by Dill &Costill (14).

Concentrations of plasma acylated ghrelin (SPI BIO, Montigney le Bretonneux, France), total PYY (Millipore, Watford, UK), leptin (R and D Systems Europe Ltd., Abingdon, UK) & insulin (Mercodia, Uppsala, Sweden) were determined using enzyme-linked immunosorbant assay kits. The associated within batch co-efficient of variation for the assays were as follows: acylated ghrelin (7.8%), leptin (6.3%), insulin (3.5%) & total PYY (7.1%).

Statistical Analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) software version 21.0 for Windows. Two-way repeated measures ANOVA were used to examine responses over time for appetite regulatory hormones and appetite perceptions. Where significant differences were found these were explored using post hoc analysis using the Bonferroni correction for multiple comparisons. When significant main effects were found area under the curve was calculated using the trapezoid method. Statistical significance was accepted at the 5% level. Repeated measures ANOVA (trial x time) showed no differences in plasma volume within (P = 0.504) or between (P = 0.834) trials therefore unadjusted plasma hormone concentrations are presented. Results are presented as Mean ± SD unless stated otherwise.

The sample size for this investigation was determined using data derived from the authors' previous research which detected compensatory acylated ghrelin responses to food restriction (31). Based on total trial AUC data (control vs. food restriction), with alpha set at 5%, beta at 80%, and a previously observed mean difference and standard deviation of 315 and 260 $pg \cdot mL^{-1} \cdot 9 \cdot^{-1}$ - it was determined that at least eight

227 particpants were required to provide sufficient statistical power for the present
228 investigation.
229
230 **Results**
231 *Exercise Responses*
232 The 90 min run undertaken on day one was completed at 11.1 ± 1.7 $km \cdot h^{-1}$ which
233 elicited 67.8 ± 4.3% of participants' maximum oxygen uptake. This induced a net
234 energy expenditure of 4908 ± 523 kJ which was derived predominantly from
235 carbohydrate oxidation rather than fat (74 ± 14 vs. 26 ± 14%). A reported RPE value
236 of 15 ± 1 indicated that participants perceived the run to be 'hard'.

237 *Appetite Hormone Responses*
238 On the morning of day two plasma acylated ghrelin concentrations were no different
239 between the exercise and control trial (P = 0.56) (Figure 2 upper panel). Two-way
240 repeated measures ANOVA (trial x time) revealed significant time (P < 0.001) and
241 interaction (P = 0.009) main effects for acylated ghrelin indicating divergent changes
242 over time between trials. Following correction for multiple comparisons using the
243 Bonferroni method no differences at individual time points were found. Further
244 analysis of the acylated ghrelin AUC identified significantly reduced levels (14%) on
245 the exercise trial following consumption of the second test meal at 4 h (Table 1). At
246 baseline on day two the fasting plasma concentration of total PYY was no different
247 between the exercise and control trial (Figure 2 lower panel). Two-way repeated
248 measures ANOVA (trial x time) revealed no differences between trials (all P > 0.05).
249
250 On day two, baseline circulating levels of plasma leptin were significantly lower on
251 the exercise trial compared with control (P = 0.03) (Figure 3 lower panel). For

252 circulating leptin, two-way repeated measures ANOVA (trial x time) revealed
253 significant trial (P = 0.016), time (P < 0.001) and interaction (P = 0.009) main effects.
254 After correction for multiple comparisons using the Bonferroni method no differences
255 were found at individual time points between trials. The plasma leptin AUC showed
256 significantly reduced circulating levels across the entirety of day two (Table 1). At
257 baseline on day two fasting plasma concentration of insulin were no different
258 between the exercise and control trial (Figure 3 upper panel). Two-way repeated
259 measures ANOVA (trial x time) revealed no differences for plasma insulin (all P >
260 0.05).
261
262 *Appetite Responses*
263 There were no significant differences in fasting appetite perceptions on day two
264 (hunger, fullness, satisfaction and PFC) between the exercise and control trial (all P >
265 0.05) (Figure 4). For each appetite perception two-way repeated measures ANOVA
266 (trial x time) revealed a main effect of time (all P < 0.001) representing changes in
267 response to test meals. However, no significant trial (all P > 0.05) or interaction (all
268 P > 0.05) main effects were found.
269
270 **Discussion**
271 Several studies have shown that there are no acute compensatory changes in
272 appetite or appetite regulatory hormones on the day during which an acute bout of
273 exercise is performed (6, 10, 31). This investigation extended the period of
274 observation in order to determine whether compensatory changes in appetite
275 regulatory parameters may occur after a time delay, namely, on the day after
276 exercise. We hypothesised that meal stimulated acylated ghrelin (suppression) and

PYY (elevation) responses would be attenuated on the day after exercise whilst circulating levels of leptin would be reduced. Furthermore, we thought that these changes would be associated with higher subjective ratings of appetite. In contrast to our hypothesis, the novel findings from this study are that acute exercise did not lead to compensatory fasting or prandial acylated ghrelin, total PYY or subjective appetite responses on the day after exercise. Paradoxically, circulating levels of acylated ghrelin were actually lower following a lunch time meal consumed 24 h after the end of exercise. In addition to these novel outcomes, this study has also re-affirmed previous findings documenting a delayed reduction in circulating leptin after a single bout of exercise with a large associated energy deficit (17, 45, 53).

Within the acute appetite regulatory system acylated ghrelin remains unique as the only circulating peptide that stimulates appetite and eating. Specifically, on a meal to meal basis, levels of acylated ghrelin rise and fall in timing with prandial changes in hunger, a pattern suggesting an important role in regulating meal initiation and/or termination (12, 13). Alongside this acute action, significant attention has also been given to understanding the extended role that acylated ghrelin plays within the regulation of energy balance and body weight. In this scenario it has been shown that acylated ghrelin responds dynamically to changes in energy balance with increases in circulating levels during periods of energy deficit being a key homeostatic response serving to defend body weight (19, 36). In the present investigation we hypothesised that exercise completed on day one would lead to higher circulating levels of acylated ghrelin on day two as a counter regulatory response to the energy deficit. Conversely, on day two, we saw no changes in circulating levels of acylated ghrelin at rest or in response to the morning test meal.

Interestingly however, after consumption of the second test meal consumed at lunch, circulating levels of acylated ghrelin were actually lower on the exercise trial.

In an exercise context, previous studies have described an attenuated postprandial acylated ghrelin response, i.e. a less marked suppression, after individuals have completed multiple bouts of exercise across several days (23, 39). This physiological change reflects an impaired satiety response and in theory would be associated with a more rapid onset of subsequent eating and potentially a greater energy intake at meals. It is not entirely clear why the findings differed in the present investigation. In the studies of Hagobian et al (23) and Mackelvie et al (39) it is likely that the attenuated meal related change in acylated ghrelin reflects the accumalated energy deficit created over several days. The present investigation studied the more short-term impact of a single bout of exercise on acylated ghrelin and this difference may explain the divergent finding. Nonetheless, the documented reduction in acylated ghrelin after the second test meal on day two was an unexpected finding and is difficult to explain given the pleotropic role of ghrelin and its complex regulation. For example, the change could be related to effects on acylated ghrelin production, secretion and/or acylation, brought about by hormonal, neural or nutritive stimuli (2, 20, 21, 22). What is clear however is that this response was unrelated to appetite as none of the subjective perceptions assessed responded to the intervention and there were no associations between acylated ghrelin and these outcomes. Further research is needed to help understand this particular finding because the existence of a delayed acylated ghrelin suppression may be meaningful.

PYY is an anorectic peptide secreted primarily by the distal intestine in response to nutrient intake (1, 3). Circulating levels of PYY typically peak 1-2 h postprandially in relation to the energy and macronutrient content of the meal with levels remaining elevated for several hours (5, 37). PYY has a critical role in the short term regulation of energy intake due to its important role in promoting satiation, satiety and delaying gastrointestinal transit (3, 4, 38). A more long term influence of PYY on energy homeostasis has also been suggested by associations that have been found between PYY, substrate oxidation and resting metabolic rate (25, 48).

Short-term food restriction (11, 31) and reductions in body weight (16) have each been shown to lower fasting and/or postprandial circulating levels of PYY. This response is likely to be part of an adaptive mechanism defending energy homeostasis. The impact of exercise on circulating PYY has been examined in several studies with the consensus suggesting that exercise transiently elevated levels of PYY (9, 47). A potential limitation of the present study was that circulating levels of total PYY were measured rather than those of $PYY_{3-36.}$ The latter variant is the modified peptide that confers the specific inhibitory effect of PYY on appetite, and although the two correlate well (50), it is possible that PYY_{3-36} may have responded differently to the intervention. Despite this, the present study is the first to characterise prandial total PYY responses on the day following an acute bout of exercise. Specifically, we examined whether an acute energy deficit induced by exercise would reduce fasting and/or postprandial levels in the circulation on the following day. The results clearly show that exercise on the prior day had no impact on plasma total PYY and these findings therefore demonstrate that total PYY is not sensitive to exercise-induced energy deficits of this magnitude within this time-frame.

In the present investigation one of the most marked changes induced by exercise was a decrease in circulating levels of leptin on the day afterwards. Specifically, in the exercise trial fasting plasma concentrations on day two were a third lower compared with control. Furthermore, across the whole of the day, circulating levels of leptin were reduced by 20% (total trial AUC) after having completed exercise. These data confirm previous reports which have documented reductions in leptin in response to acute exercise. Notably, the consensus arising from previous work, and supported here, are that substantial reductions in circulating leptin occur after exercise when associated with sufficiently high energy expenditure (> 3348 kJ) and following a latency period of ~24- 48 h (17, 45, 53). Existing work has shown that circulating levels of leptin are highly responsive to alterations in energy balance/availability (8, 26) and therefore the change observed in the current study is likely to be related to the energy deficit imposed by exercise (~ 5020 kJ) which was maintained going forward into day two due to strict dietary and physical activity control. It is perhaps interesting to note that comparatively the magnitude of this decrease in leptin is approximately half of that which occurs in response to fasting over a similar period (35). The change seen with exercise in this study therefore reflects the less severe perturbation to energy balance.

In concert with leptin, insulin also functions as a chronic regulator of energy homeostasis, providing information to the central nervous system regarding stored energy within adipose tissue (52). Unlike leptin however, in the short-term, insulin is also a critical regulator of circulating glucose and responds dynamically to systemic perturbations in glycaemia. Additionally, both fasting and postprandial insulin concentrations are mediated at a higher level by insulin sensitivity within peripheral

tissues, such as skeletal muscle, liver and adipose tissue. Acutely, perhaps the most significant and well characterised impact that exercise has on insulin is a reduction in circulating levels that occur secondary to improvements in peripheral tissue sensitivity that can last for up to 48 h post exercise (24). In the present study we did not detect any changes in insulin either when fasted or postprandially. Thus, in the context of the present study the exercise/energy deficit did not manifest as an alteration in circulating insulin. The lack of change in insulin within this study likely reflects the fact that the particpants examined were young, lean and healthy, with no capacity of exercise to enhance insulin sensitivity further.

The effect of exercise on subjective appetite perceptions has received widespread attention within psycho-biological research over the last 20 years. The most consistent finding within this body of literature is that single bouts of exercise transiently suppress appetite, a phenomena that has been termed exercise induced anorexia (32). This effect is brief, typically lasting no more than 30 min, and does not typically affect food intake when measured for several hours afterwards (29, 30). This response to an exercise-induced energy deficit is in direct contrast to that observed when food restriction is used as a method to induce negative energy balance. In this scenario, rapid and marked compensatory increases in appetite and food intake are noted (27, 31). Although in the immediacy a rather loose coupling exists between exercise induced energy expenditure, appetite and food intake, one study has suggested that an association may begin to emerge after a delay of approximately two days (15). In the present investigation we sought to explore this relationship further within a controlled laboratory setting by assessing changes in subjective appetite parameters on the day after exercise. In this study, at no point

within day two did exercise affect subject ratings of hunger, fullness, satisfaction or prospective food consumption. These results are consistent with those from a previous investigation with a similar study design, participant group and exercise-induced energy deficit (34). Clearly, a period of negative energy balance cannot continue indefinitely, and although reductions in energy expending processes are expected to occur, at some point it is likely that a compensatory increase in appetite will manifest. For the current study population it would seem that this lag phase endures for more than 24 h, however further research is needed to determine the exact time-scale of this response.

In conclusion this study has shown that a large (4908 ± 523 kJ) exercise induced energy deficit leads to a compensatory decrease in circulating levels of leptin on the day afterwards. Conversely, circulating levels of acylated ghrelin, total PYY and subjective appetite perceptions do not display counter regulatory responses within this time-frame. Interestingly, exercise actually led to a reduction in circulating levels of acylated ghrelin in the afternoon on the day following exercise. These data suggest that short acting appetite regulatory hormones do not couple strongly to exercise induced energy deficits within the 24 h after exercise. Instead, exercise-induced perturbations in energy balance of this magnitude manifest within this time-frame as a notable reduction in circulating leptin. This physiological change shows that exercise induced energy deficits are initially sensed within 24 h however the lack of change in subjective appetite perceptions suggests that this signal does not reach consciousness at this time.

Acknowledgments

The research was supported by the National Institute for Health Research (NIHR) Diet, Lifestyle & Physical Activity Biomedical Research Unit based at University Hospitals of Leicester and Loughborough University. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Author Contributions

JAK and MAN conceived the study. JAK, JOG, BMK and SX performed the experimental procedures. APJ, JAK and SX conducted the biochemical analysis. JAK and MAN wrote the manuscript. All authors reviewed the final version of the manuscript before submission.

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Figure Legends

Figure 1

Schematic illustration of the main trial protocol

Figure 2

Plasma acylated ghrelin (upper panel) & PYY (lower panel) concentrations in the control (♦) and exercise (■) trials. For clarity values are mean ± SEM, *n* = 9. Black boxes represent test meals.

Figure 3

Plasma insulin (upper panel) & leptin (lower panel) concentrations in the control (♦) and exercise (■) trials. For clarity values are mean ± SEM, *n* = 9. Black boxes represent test meals.

Figure 4

Subjective ratings of hunger (top left), prospective food consumption (top right), fullness (bottom left) and satisfaction (bottom right) in the control (♦) and exercise (■) trials. For clarity values are mean ± SEM, *n* = 9. Black boxes represent test meals.

Table 1: Day two circulating acylated ghrelin and leptin area under the concentration-time curve profiles

	Total Trial (0-7 h)			Test Meal 1 Response (0-4 h)			Test Meal 2 Response (4-7 h)		
	units 7 h			*units 4 h*			*units 3 h*		
Acylated Ghrelin									
Control	698	±	298	371	±	166	326	±	136
Exercise	623	±	312	344	±	179	279	±	136*****
Leptin									
Control	7266	±	3949	3697	±	3068	3570	±	2006
Exercise	5841	±	3335*****	3068	±	1626*****	2773	±	1725*****

Values are pg·mL·unit time and ng·mL·unit time for acylated ghrelin and leptin (mean ± SD, n = 9). *different from control ($P < 0.05$)