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Appetite

Research report

Appetite, appetite hormone and energy intake responses to two consecutive days of aerobic exercise in healthy young men ☆

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ABSTRACT

Single bouts of exercise do not cause compensatory changes in appetite, food intake or appetite regulatory hormones on the day that exercise is performed. It remains possible that such changes occur over an extended period or in response to a higher level of energy expenditure. This study sought to test this possibility by examining appetite, food intake and appetite regulatory hormones (acylated ghrelin, total peptide-YY, leptin and insulin) over two days, with acute bouts of exercise performed on each morning. Within a controlled laboratory setting, 15 healthy males completed two, 2-day long (09:00–16:00) experimental trials (exercise and control) in a randomised order. On the exercise trial participants performed 60 min of continuous moderate-high intensity treadmill running (day one: $70.1 \pm 2.5\%$ VO$_2$peak, day two: $70.0 \pm 3.2\%$ VO$_2$max (mean $\pm$ SD)) at the beginning of days one and two. Across each day appetite perceptions were assessed using visual analogue scales and appetite regulatory hormones were measured from venous blood samples. Ad libitum energy and macronutrient intakes were determined from meals provided two and six hours into each day and from a snack bag provided in-between trial days. Exercise elicited a high level of energy expenditure (total = 7566 ± 635 kJ across the two days) but did not produce compensatory changes in appetite or energy intake over two days (control: $29,217 \pm 4006$ kJ; exercise: $28,532 \pm 3899$ kJ, $P > 0.050$). Two-way repeated measures ANOVA did not reveal any main effects for acylated ghrelin or leptin (all $P > 0.050$). However a significant main effect of trial ($P = 0.029$) for PYY indicated higher concentrations on the exercise vs. control trial. These findings suggest that across a two day period, high volume exercise does not stimulate compensatory appetite regulatory changes.

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Introduction

The interaction between exercise, appetite control and energy balance has direct relevance for the implementation of exercise as a therapeutic strategy for weight control. Over the last decade, many studies have investigated the impact of acute exercise, and exercise training, on appetite perceptions, food intake and energy balance (Hopkins, King, & Blundell, 2010). Currently, a disparity exists whereby on the one hand evidence suggests that exercise training (4–12 months) does not induce substantial weight loss due to compensatory responses i.e. increases in appetite and food intake

(Hopkins et al., 2014; King et al., 2007; Wing, 1999); whilst on the other hand acute laboratory-based studies consistently document that acute exercise has no influence on appetite or ad libitum food intake (King, Burley, & Blundell, 1994; King, Wasse, & Stensel, 2013; Wasse, Sunderland, King, Batterham, & Stensel, 2012). Indeed, a recent meta-analysis concluded that acute exercise has a trivial effect on subsequent energy intake (Schubert, Desbrow, Sabapathy, & Leveritt, 2013). It is possible that this inconsistency between outcomes is related to the rather loose coupling which exists between perturbations to energy balance induced by exercise and subsequent energy intake (Blundell & King, 1998). Compensatory alterations in appetite and food intake in response to single bouts of exercise could possibly occur over a longer duration than that previously examined in acute laboratory studies i.e. over more than 24 h. To date, only two studies have looked at this proposition in detail under strict laboratory conditions. In one study, appetite responses before and after a test meal were examined on the day after a single bout of moderate-intensity exercise (Heden, Liu, Park, Dellsperger, & Kanaley, 2013). The researchers reported that

exercise on the previous evening reduced perceptions of fullness in obese volunteers, but had no influence in lean individuals. More recently, Beaulieu, Olver, Abbott, and Lemon (2015) examined appetite and *ad libitum* energy intake responses over 34 h with an acute bout of sprint interval exercise being performed at the beginning of the trial. The researchers observed a transient suppression of appetite, characteristic of exercise-induced anorexia (King et al., 1994), but then observed a latent increase in hunger and motivation to eat several hours after exercise leading to increased 34-h AUC values compared to control conditions. There was no effect of exercise on energy intake. However, it should be noted that in this study appetite perceptions and *ad libitum* energy intake may have been compromised by reports of nausea and vomiting during and after exercise (Seimon et al., 2010). Additional work is needed to extend these preliminary findings in order to identify the initial stage of compensation and to better understand the regulatory mechanisms.

Advancements in knowledge about the neuro-humoural regulation of appetite and energy homeostasis have catalysed research investigating the influence of exercise on hormones implicated in appetite control and energy balance (King, Wasse, Stensel, & Nimmo, 2013; Schubert, Sabapathy, Leveritt, & Desbrow, 2013; Stensel, 2010). Findings from this body of work were recently pooled in a meta-analysis which concluded that acute exercise has a small to moderate effect on appetite hormones, suppressing acylated ghrelin, and stimulating peptide-YY (PYY), glucagon-like-peptide-1 (GLP-1) and pancreatic polypeptide (Schubert, Sabapathy et al., 2013). These alterations are transient, and often coincide with appetite loss during and after moderate to high intensity exercise (however there is often little effect on subsequent changes in food intake). Unfortunately, at present, there is a dearth of information regarding the more protracted effects of exercise on these important mediators of appetite control. As an exception, Heden et al. (2013) observed attenuated fasting and post-meal suppression of acylated ghrelin after a standardised breakfast meal in lean individuals on the morning after exercise the prior evening. This response is suggestive of a compensatory mechanism serving to defend energy balance i.e. less effective suppression of an appetite stimulating signal. No changes in acylated ghrelin were seen in obese individuals. Unfortunately in this study energy intake was not examined on the day after exercise; thus future research is required to identify if the highlighted changes in acylated ghrelin influence subsequent energy intake. It has been hypothesised that compensatory responses in appetite regulatory hormones and energy intake may be delayed, and future research is needed to investigate this by examining longer term responses to energy imbalance.

The present investigation sought to advance previous research by characterising the impact of exercise, performed on two consecutive mornings, on appetite, *ad libitum* food intake and key hormones implicated as important regulators of appetite and eating (acylated ghrelin, PYY, leptin and insulin). Specifically, by eliciting a marked perturbation to energy homeostasis by performing two prolonged bouts of moderate-high intensity treadmill running, we imposed a notable and unfamiliar stimulus (for the participants) through which to assess the impact of exercise in controlled conditions over a more prolonged period than typically implemented. Based on previous findings, in response to this stimulus, we hypothesised that appetite and energy intake would be unaltered on the first day of exercise, but that on the second day, compensatory increases in appetite, energy intake and acylated ghrelin would be seen. Conversely, compensatory decreases in circulating PYY and leptin may be apparent.

Methods

Participants

Following approval from the Institutional Ethics Advisory Committee, 15 healthy physically active males (18–24 years) provided

Table 1
Characteristics of the study participants ($N = 15$).

Characteristic	Mean ± SD
Age (years)	21.1 ± 1.7
BMI (kg·m^{-2})	23.0 ± 1.9
Body mass (kg)	74.8 ± 6.8
Waist circumference (cm)	78.5 ± 5.2
Treadmill peak oxygen uptake (mL·kg^{-1}·min^{-1})	57.9 ± 4.2

written informed consent to participate in this study. Participants were non-smokers, not taking medication, weight stable for at least three months before the study (body mass deviation of less than ± 2 kg) and were not dieting or exhibiting any extreme dietary habits (including disinhibited and restrained eating tendencies) (Stunkard & Messick, 1985). Participants had no history of cardiovascular or metabolic disease. Participants were recreational games players, not accustomed to performing repeated, prolonged bouts of moderately-high intensity running. This ensured that the aerobic exercise stimulus was unfamiliar to the participants. Table 1 describes the participant characteristics.

Preliminary testing

Before main trials, participants visited the laboratory to undergo preliminary assessments (questionnaires, anthropometry, exercise testing) and to be familiarised with the laboratory environment and study procedures. Specifically, participants completed questionnaires assessing health status, food preferences, habitual physical activity and psychological eating tendencies (Three-Factor Eating Questionnaire (TFEQ)) (Stunkard & Messick, 1985). Participants scoring 11 or more for any factor on the TFEQ were determined to exhibit restrained or disinhibited eating tendencies, and consequently excluded from taking part. Height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany) and body weight was measured to the nearest 0.01 kg using a balance beam scale (Avery Industrial Ltd, Leicester, UK). Body mass index (BMI) was subsequently calculated as weight (kg) divided by height (m²). Waist circumference was determined as the narrowest part of the torso between the xiphoid process and the iliac crest (Ross et al., 2008).

To determine the treadmill speed required to elicit 70% of maximum aerobic capacity during main trials, two preliminary running tests were undertaken; 1) a progressive-submaximal test to determine the relationship between treadmill speed and oxygen consumption, 2) a peak oxygen uptake test. Both tests have been described in-depth previously (Broom, Stensel, Bishop, Burns, & Miyashita, 2007). During each test, oxygen consumption and carbon dioxide production were determined from frequent expired air samples collected into Douglas bags (Plysu Protection Systems, Milton Keynes, UK) and analysed using a dry gas metre (Harvard Apparatus, Edenbridge, Kent, UK) and paramagnetic oxygen/infra-red carbon dioxide analyser (Series 1400, Servomex, Crowborough, East Sussex, UK).

Experimental procedures

Participants completed two, 2-day experimental trials (exercise and control) in a crossover design with each trial being separated by at least one-week (Fig. 1). Each trial required participants to attend the laboratory between 09:00 and 16:00 on day one and day two. Participants remained physically inactive in the time spent away from the laboratory in-between days one and two. *Ad libitum* food intake was monitored throughout trials including the evening period between days one and two.
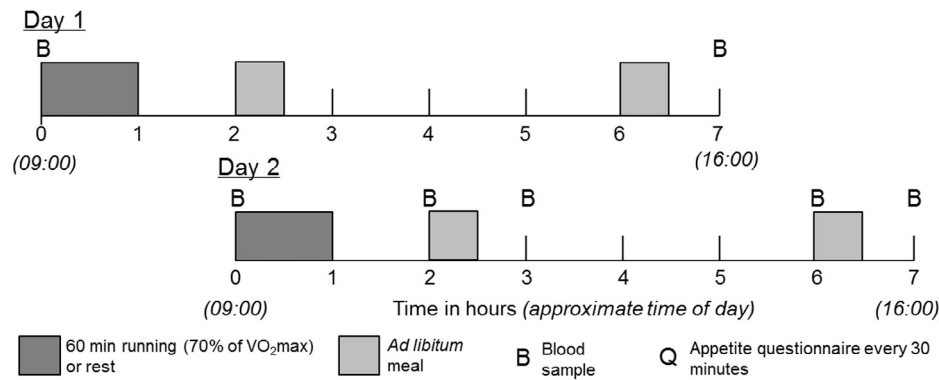
Fig. 1. Schematic representation of the main trial protocol.

Before each trial participants standardised their dietary intake for 48 h using a weighed food record (24-h average reported energy intake: 11,226 ± 2956 kJ). Alcohol, caffeine and structured physical activity were not permitted during this time. On day one of the exercise trial participants arrived at the laboratory following an overnight fast of at least 10 h. After the collection of a fasting blood sample, participants commenced a 60 min continuous treadmill run (Technogym Excite Med, Cesena, Italy) at a speed predicted to elicit 70% of VO_2 peak. Expired air samples were collected at 15, 30, 45 and 60 min during exercise to monitor the intensity with subtle adjustments being made to the treadmill speed if necessary. Heart rate and ratings of perceived exertion (RPE, Borg, 1973) were assessed periodically during the run. Energy expenditure during the exercise session was calculated from oxygen uptake and carbon dioxide production (Frayn, 1983). These measurements were also used to calculate non-protein respiratory exchange ratio (RER) (carbon dioxide production divided by oxygen consumption).

Following the run participants rested in the laboratory for 6 h (sitting, reading, working at a desk or watching films). At the end of this time a blood sample was collected, after which participants left the laboratory. Participants returned the next morning at 09:00 (fasted) to commence day two of the trial. In the time spent away from the laboratory participants remained inactive (<5000 steps; Tudor-Locke & Bassett, 2004) and this was confirmed by a pedometer (Yamax DIGI-WALKER SW200, San Antonio, USA) which each participant wore. Day two of main trials was identical to day one except that blood samples were collected more frequently (five samples on day 2 compared with two samples on day 1) to measure circulating concentrations of appetite regulatory hormones (see Fig. 1). The procedures in the control trial were identical to the exercise trial except that no exercise was performed. The sole exception to this was that resting expired air samples were collected during the first hour of each control trial day to assess resting metabolic rate. These data subsequently permitted the calculation of net exercise energy expenditure i.e. gross exercise energy expenditure minus resting energy expenditure.

Appetite and food intake

During the laboratory phase of the main trials appetite perceptions (hunger, satisfaction, fullness and prospective food consumption (PFC)) were assessed at baseline and every 30 min thereafter using 100 mm visual analogue scales (Flint, Raben, Blundell, & Astrup, 2000). Energy and macronutrient intake were assessed from two *ad libitum* meals provided on both days of the exercise and control trials. Specifically, at 2 h a cold buffet meal was provided with participants being given access for 30 min. The items available were: semi-skimmed milk, three varieties of cereal, white bread, brown

bread, margarine, mayonnaise, ham, tuna, crisps, chocolate rolls, Mars bars, cereal bars, cookies, muffins, apples, oranges and bananas. The buffet foods were presented identically on each occasion and were in excess of expected consumption. Participants were instructed to eat until 'comfortably full' and informed that additional food was available if desired. At 6 h a hot *ad libitum* meal was provided. This was a homogenous pasta meal, composed of white fusilli that was cooked in a microwave for 10 min in unsalted water and mixed with a tomato sauce, Cheddar cheese and olive oil. The macronutrient composition of the meal was balanced (52% carbohydrate, 14% protein and 34% fat), and designed to meet current UK dietary guidelines (Food Standards Agency, 2007). Participants were provided with a small bowl of the meal which was replaced with a full bowl at regular intervals in an attempt to blind the participants from the amount of food eaten. Participants consumed meals in isolation so that social influence did not affect food selection or quantity eaten. Leftovers were weighed and food consumption was determined as the weighed difference of items before and after each meal. The energy and macronutrient content of the items consumed were determined from manufacturers' values.

For the time spent away from the laboratory in-between the visits on day one and day two participants were free to select, and consume, a selection of items presented at the cold buffet meal. Participants were permitted to consume these items after leaving the laboratory on day one and until 22:30. The food items available for selection included; crisps, chocolate rolls, Mars bars, cereal bars, cookies, muffins, apples, oranges and bananas. Leftovers were returned the next day to determine actual consumption. Water was available *ad libitum* throughout trials.

Blood sampling

Venous blood samples were collected at 0 and 7 h on day one of each trial, and at 0, 2, 3, 6 and 7 h during day two of trials. Both samples on day one, and the baseline sample on day two, were collected by venepuncture of an antecubital vein. The remaining blood samples on day two were collected using a cannula (Venflon, Becton Dickinson, Helsingborg, Sweden) inserted into an antecubital vein. Blood samples were taken into pre-chilled EDTA monovettes (Sarstedt, Leicester, UK) for the measurement of plasma total PYY, acylated ghrelin, leptin, glucose and insulin. As per manufacturer instructions, samples collected for acylated ghrelin were taken into monovettes containing protease inhibitors and were spun in a refrigerated centrifuge (Heraeus Labofuge 400R, Thermo Electron, Osterode, Germany) at $1287 \times g$ for 10 min at 4 °C. The plasma supernatant was immediately removed and acidified before sample storage. All other samples were spun at $1681 \times g$ for 10 min at 4 °C

with plasma supernatant being collected and stored for future analysis.

At each sampling point duplicate 20 μL blood samples were collected into micropipettes for the determination of haemoglobin, and triplicate 20 μL blood samples were collected into heparinised micro haematocrit tubes for the determination of blood haematocrit concentration. These data were used to estimate plasma volume and to assess changes over time (Dill & Costill, 1974).

Biochemical analysis

Enzyme immunoassays were used to determine concentrations of plasma acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total PYY (Millipore, Billerica, USA), leptin (R&D Systems, Minneapolis, USA) and insulin (Mercodia, Uppsala, Sweden). Plasma glucose concentrations were determined by enzymatic, colorimetric methods using a bench top analyser (Pentra 400; HORIBA ABX Diagnostics, Montpellier, France). To eliminate inter-assay variation, samples from each participant were analysed on the same run. The within-batch coefficients of variation for the assays were as follows: acylated ghrelin 10.9%, total PYY 7.7%, leptin 5.9%, insulin 7.9%, and glucose 0.6%.

Statistical analysis

Data were analysed using IBM SPSS statistics, version 21.0 for Windows. Appetite perceptions and appetite hormone area under the curve (AUC) calculations were performed using the trapezoidal method. Comparison between trials for exercise responses, energy and macronutrient intake, and day two AUC appetite hormone data were compared using Student's paired t-tests. Two-way repeated measures ANOVA was used to examine differences between trials over time for appetite perceptions, energy and macronutrient intake and appetite hormones. Where significant trial and interaction effects were found, *post-hoc* analysis was performed using Holm–Bonferroni correction for multiple comparisons (Atkinson, 2002). The Pearson product moment correlation coefficient was used to examine relationships between variables. Corrections of plasma analyte values for changes in plasma volume did not alter the statistical significance of the results; therefore, for simplicity, the unadjusted values are presented. Statistical significance was accepted at 5%. Results are shown as mean ± SD in text and tables, and mean ± SEM in figures (for clarity).

Results

Exercise responses

The exercise responses for day one and day two are summarised in Table 2. There was no significant difference in oxygen consumption, exercise intensity, running speed, RPE, net energy expenditure or respiratory exchange ratio (RER) between the two days of exercise. There was no difference between trials in pedometer-assessed

Table 2
Exercise responses on days one and two.

Response	Day one	Day two	P
Running speed (km·h^{-1})	11.9 ± 1.0	11.9 ± 1.0	–
VO$_2$ (mL·kg^{-1}·min^{-1})	40.6 ± 3.1	40.5 ± 3.5	0.87
Exercise intensity (% of VO$_2$ peak)	70.1 ± 2.5	70.0 ± 3.2	0.85
RPE (6–20)	15 ± 2	15 ± 2	0.62
Net energy expenditure (kJ)	3779 ± 327	3787 ± 327	0.85
RER	0.95 ± 0.04	0.93 ± 0.03	0.17

Mean ± SD (N = 15). VO$_2$, oxygen uptake; RER, non-protein respiratory exchange ratio; RPE, rating of perceived exertion; VO$_2$ peak, peak oxygen uptake.

physical activity (control 4337 ± 1683; exercise 4524 ± 2112 counts $P = 0.991$, $r = 0.84$) demonstrating that participants remained equally inactive outside of formal exercise periods during main trials (<5000 steps; Tudor-Locke & Bassett, 2004).

Appetite responses

Fasting appetite perceptions (hunger, satisfaction, fullness and PFC) did not differ between trials at baseline on either day of each trial (all $P > 0.061$). Two-way ANOVA revealed a significant main effect of time (all $P < 0.001$) and interaction effects (all $P < 0.001$) for each appetite perception (Fig. 2). Significant trial effects were also reported for hunger ($P = 0.046$) and PFC ($P = 0.045$), indicating suppressed levels in the exercise trial across days one and two. Compared with control, *post-hoc* analysis revealed suppressed hunger ratings at 1.5 h on day one ($P = 0.029$) and at 0.5 ($P = 0.029$) and 1 h ($P < 0.001$) on day two of the exercise trial. Fullness ratings were significantly elevated at 1 h on day one of the exercise trial ($P = 0.030$), whilst PFC was lower at 0.5 ($P = 0.029$) and 1 h ($P < 0.001$) on day two of the exercise trial.

Analysis of the appetite AUC (calculated separately for days one and two) using two-way ANOVA (day vs. trial) confirmed significant trial effects for hunger ($P = 0.042$), fullness ($P = 0.050$), and PFC ($P = 0.036$) but did not show any differences in appetite between trial days one and two (data not shown).

Tables 3 and 4 show the energy and macronutrient intake data from the exercise and control trials. No significant main effects were apparent for energy intake (all $P > 0.137$). For macronutrient intake, no significant main effects were found for carbohydrate or fat intake (all $P > 0.261$), however a main effect of time was observed for protein intake ($P < 0.001$) which was reduced during the evening period between days 1 and 2 on both the control and exercise trials. Relative energy intake (REI) was calculated for days one and two as energy intake minus the net energy expenditure elicited by exercise (exercise day one: 3179 ± 586 kJ; exercise day two: 1934 ± 321 kJ; control day one: 4127 ± 663 kJ; control day two: 2856 ± 196 kJ). Significant trial ($P < 0.001$) and time ($P < 0.001$) effects were found, indicating lower REI across days one and two on the exercise trial and also reflecting the fewer number of meals consumed on day two compared with day one (three vs. two) on both trials.

Two-way ANOVA revealed a significant main effect of trial for *ad libitum* water intake ($P < 0.001$) (exercise day one: 1825 ± 825 mL; exercise day two: 1687 ± 692 mL; control day one: 1319 ± 842 mL; control day two: 1301 ± 591 mL).

Blood analyses

Due to problems with blood sampling data for plasma acylated ghrelin, total PYY and leptin are presented for 14 (rather than 15) participants whilst data for plasma insulin are presented for 13 (rather than 15) participants. Figure 3 shows the circulating appetite hormone responses on days one and two of the control and exercise trials.

Fasting acylated ghrelin concentrations did not differ between the exercise and control trials at baseline on day one or day two (control day one: 128 ± 113; exercise day one: 144 ± 169; control day two: 127 ± 121; exercise day two: 117 ± 143 pg/mL (P for trial, time and interaction all >0.05)) (Fig. 3A). Across the whole trial, two-way repeated measures ANOVA did not show any significant main effects for plasma acylated ghrelin (all $P > 0.05$).

Fasting plasma total PYY concentrations did not differ between trials at baseline on day one or day two of main trials (control day one: 84 ± 40; exercise day one: 95 ± 63; control day two: 87 ± 37; exercise day two: 104 ± 50 pg/mL (P for trial, time and interaction all >0.05)) (Fig. 3B). Across the whole trial, two-way repeated measured ANOVA revealed a significant main effect of trial ($P = 0.029$)
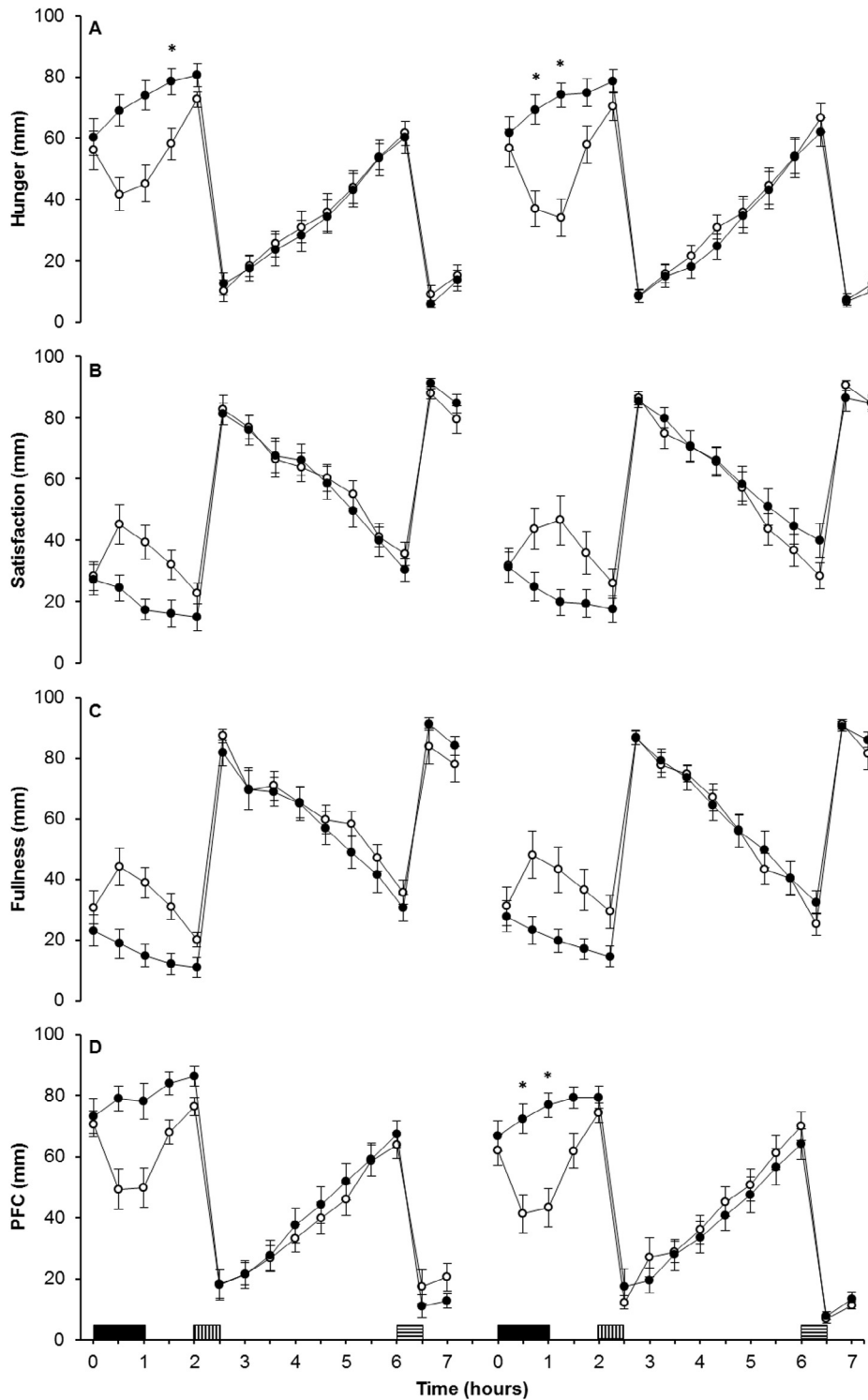
Fig. 2. Perceptions of hunger (A), satisfaction (B), fullness (C) and prospective food consumption (D) in control (●) and exercise (○) trials. Values are mean ± SEM, N = 15. Black rectangles indicate exercise, vertically shaded rectangles indicate cold buffet meals, and horizontally shaded rectangles indicate hot buffet meals. *Significant difference between control and exercise (P < 0.03).

indicating higher total PYY concentrations on the exercise trial. There was also a significant main effect of time (P < 0.001) for total PYY.

Fasting plasma leptin concentrations did not differ between trials at baseline on day one or day two of the exercise and control trials (control day one: 1543 ± 1042; exercise day one: 1738 ± 1716; control day two: 1860 ± 2044; exercise day two: 1710 ± 1733 pg/mL, P > 0.333) (Fig. 3C). Across trial days, two-way repeated measures

ANOVA revealed a significant main effect of time (P < 0.001) but no trial or interaction effects (P > 0.05).

Fasting plasma insulin concentrations were found to differ across time (P = 0.0015); however, there were no trial or interaction effects (both P > 0.05) (control day one: 21 ± 5; exercise day one: 25 ± 10; control day two: 30 ± 10; exercise day two: 29 ± 13 pmol/mL) (Fig. 3D). Across trial days, two-way repeated measures ANOVA

Table 3

Ad libitum energy intake during the control and exercise trials.

Day	Meal	Exercise (kJ)	Control (kJ)	P
1	Cold buffet (2 h)	5950 ± 1855	6053 ± 2363	0.84
	Hot buffet (6 h)	5777 ± 1407	6052 ± 902	0.43
	Overnight	5085 ± 1087	5163 ± 1214	0.80
2	Cold buffet (2 h)	5432 ± 1231	5839 ± 1587	0.39
	Hot buffet (6 h)	6288 ± 1738	6109 ± 821	0.60
	Total trial	28,532 ± 3899	29,217 ± 4006	0.45

Mean ± SD (N = 15). Food consumption outside of the lab on day 1 (overnight) was between 16:30 and 22:30.

revealed a significant trial ($P = 0.025$), time ($P < 0.001$) and interaction effect ($P = 0.019$) for plasma insulin concentrations. *Post-hoc* analysis showed insulin levels to be significantly lower for the exercise condition in comparison to the control condition at 2 h on day two ($P < 0.001$).

Area under the curve calculations for day two of each trial revealed no significant differences for acylated ghrelin (control: 833 ± 1090; exercise: 909 ± 1337 pg/mL/h) and leptin (control: 10,376 ± 10,652; exercise: 9783 ± 9244 pg/mL/h) (both $P > 0.050$). For insulin, values were significantly lower during the exercise trial (control: 1446 ± 785; exercise: 852 ± 316 pmol/mL/h, $P = 0.050$), whilst total PYY values were significantly higher (control: 720 ± 253; exercise: 883 ± 374 pg/mL/h, $P = 0.010$).

Correlations

Correlation analyses were performed for all major outcomes (AUC hormone data and appetite ratings, energy intake and subject characteristics); however no statistically significant outcomes were found (data not shown).

Table 4

Macronutrient intake in the control and exercise trials.

Day		Fat (g)	Carbohydrate (g)	Protein (g)
		[%]	[%]	[%]
	Control Trial			
1	Cold buffet (2 h)	51 ± 35	203 ± 62	45 ± 17
		[29 ± 10]	[58 ± 11]	[13 ± 4]
	Hot meal (6 h)	54 ± 8	187 ± 28	51 ± 8
		[34 ± 0]	[52 ± 0]	[14 ± 0]
	Overnight food	47 ± 14	188 ± 46	14 ± 3
		[34 ± 5]	[61 ± 6]	[5 ± 0]
2	Cold buffet (2 h)	47 ± 24	201 ± 55	43 ± 16
		[29 ± 11]	[59 ± 12]	[13 ± 5]
	Hot meal (6 h)	54 ± 7	189 ± 24	52 ± 7
		[34 ± 0]	[52 ± 0]	[14 ± 0]
	Total trial	252 ± 59	968 ± 133	205 ± 29
		[32 ± 4]	[56 ± 4]	[12 ± 2]
	Exercise Trial			
1	Cold buffet (2 h)	51 ± 28	195 ± 48	46 ± 19
		[30 ± 11]	[57 ± 11]	[13 ± 4]
	Hot meal (6 h)	54 ± 13	188 ± 44	52 ± 12
		[34 ± 0]	[52 ± 0]	[14 ± 0]
	Overnight food	47 ± 12	184 ± 43	14 ± 3
		[35 ± 5]	[60 ± 5]	[5 ± 0]
2	Cold buffet (2 h)	48 ± 24	175 ± 24	43 ± 16
		[31 ± 11]	[56 ± 12]	[13 ± 3]
	Hot meal (6 h)	58 ± 13	201 ± 45	54 ± 13
		[34 ± 0]	[52 ± 0]	[14 ± 0]
	Total trial	258 ± 45	942 ± 111	208 ± 41
		[33 ± 4]	[55 ± 4]	[12 ± 1]

Mean ± SD (N = 15). Food consumption outside of the lab on day 1 (overnight) was between 16:30 and 22:30.

Discussion

The consensus from the research literature suggests that exercise does not stimulate appetite on the day it is performed, even when responses have been examined for several hours post-exercise. Conversely, daily exercise undertaken over four to five consecutive days has been shown to increase hunger, motivation to eat or reduce satiation (Hagobian et al., 2009; Mackelvie et al., 2007). The present study sought to explore whether initial alterations in appetite could be detected after two bouts of exercise performed on consecutive days across a period of 30 h after the first bout of exercise. Besides witnessing a transient exercise-induced suppression of appetite on each day, we observed no subsequent changes in any of the subjective appetite measurements assessed. Thus, repeated bouts of exercise on two days did not impact hunger, satiation or satiety. These findings indicate that over this duration, exercise and its associated energy expenditure, are not perceived.

Previously, King, Lluch, Stubbs, and Blundell (1997) examined appetite responses across two days with two bouts of exercise being performed on the first day of the exercise trial (combined energy expenditure 4983 ± 1004 kJ). Similar to the current investigation, these researchers observed no alterations in appetite. It should however be noted that in this particular study appetite assessments were made in participants' free living environment; meaning that various extraneous factors may have confounded the results. More recently, Heden et al. (2013) also observed no changes in fasting or postprandial ratings of hunger and fullness in response to a test meal consumed on the morning after moderate-intensity exercise completed on the previous evening. In contrast to these findings, Beaulieu et al. (2015) recently published data showing a greater motivation to eat on the day after a single bout of sprint interval training (supra-maximal exercise) in a small group of trained men (N = 8). Taken collectively, the available data suggest that acute exercise, even when repeated on two days, does not automatically stimulate appetite. Very high intensity exercise may be an exception; however additional studies with a larger group of more diverse participants are needed to state this with any certainty.

An additional aim of the present investigation was to characterise the extended impact of exercise on energy and macronutrient intake. Consistent with the lack of alteration in appetite, we also observed no significant changes in energy or macronutrient intake assessed from several *ad libitum* meals consumed across days one and two. Previous studies have shown that over an extended period (one to two weeks) individuals may begin to partially increase the amount of food consumed in an attempt to counter the heightened energy expended during exercise (Stubbs, Sepp, Hughes, Johnstone, Horgan et al., 2002; Stubbs, Sepp, Hughes, Johnstone, King et al., 2002; Whybrow et al., 2008). The precise point at which this response is initiated is not known; however it is clear that such compensation does not occur immediately on the actual day that exercise is performed (Schubert, Desbrow et al., 2013). When considering responses on the day after exercise, King et al. (1997) previously reported a lack of change in energy intake. Unfortunately in this study the implementation of diaries to assess energy intake, and the aforementioned lack of study control, limits the strength of these findings. The present study sought to provide a more rigorous analysis by utilising laboratory based assessments of energy and macronutrient intake. The present results are consistent with recent findings reported by Beaulieu et al. (2015) who observed no changes in energy intake within the 36 h after sprint interval training. Together, these data indicate that the initial stages of energy intake compensation are not seen on the day after exercise, even when a successive bout is performed on the second day. It is likely that compensatory responses will begin to appear after
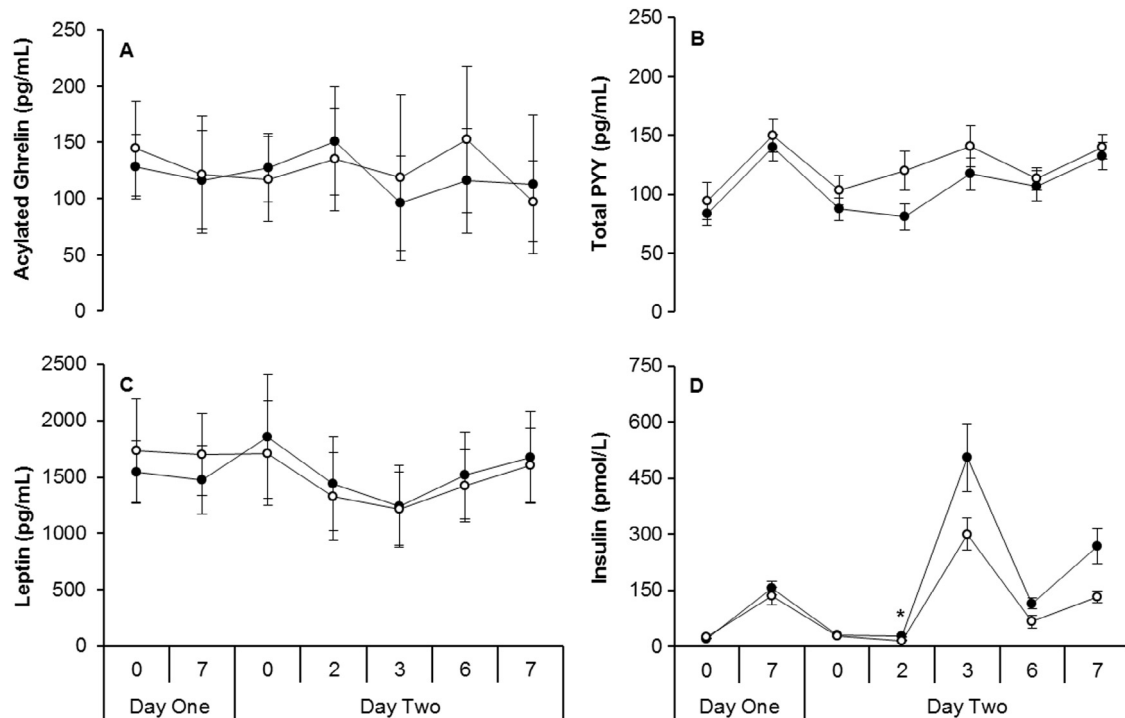
Fig. 3. Plasma concentrations of acylated ghrelin, $N = 14$ (A), total PYY, $N = 14$ (B), leptin, $N = 14$ (C), and insulin, $N = 13$ (D) in control (●) and exercise (○) trials. Values are mean ± SEM. *Significant difference between control and exercise. NB: meals were consumed at 2 and 6 h on days 1 and 2.

additional days of exercise with a higher level of energy expenditure and/or negative energy balance.

Appetite and food intake are subject to regulation by a complex network of neural-hormonal mediators which act acutely (meal to meal) and chronically (over days/weeks) to facilitate energy homeostasis (Neary & Batterham, 2009; Perry & Wang, 2012). Notable within the short-term regulation of appetite and energy intake are acylated ghrelin and PYY which work antagonistically to coordinate meal initiation and termination (De Vriese, Perret, & Delporte, 2010; Manning & Batterham, 2014). Specifically, acylated ghrelin remains unique as the only known hormonal stimulant of appetite, with circulating levels increasing prior to meal initiation and subsequently decreasing postprandially (Cummings, Frayo, Marmonier, Aubert, & Chapelot, 2004). Conversely, PYY is a key mediator of satiation and satiety, with circulating levels increasing in response to nutrient intake and remaining elevated between meals (Le Roux et al., 2005). Acylated ghrelin and PYY are also implicated in the chronic regulation of energy homeostasis with each being sensitive to deviations in energy balance/stores. Specifically, negative energy balance and weight loss increased circulating levels of acylated ghrelin (King et al., 2011; Leidy, Dougherty, Frye, Duke, & Williams, 2007) and reduced those of PYY (Hill, De Souza, & Williams, 2011; King et al., 2011). These responses serve to promote the restoration of energy balance and limit perturbations in body weight. The present study sought to characterise the latent impact of exercise on acylated ghrelin and PYY. Specifically, we wanted to determine how exercise, and the associated high level of energy expenditure, would impact circulating levels of these hormones over an extended duration. It was anticipated that fasting and/or postprandial levels of acylated ghrelin on day two would be elevated, whilst those of PYY would be reduced as mechanisms to preserve energy homeostasis. In contrast, we saw no changes in acylated ghrelin whilst PYY was transiently elevated on day two after exercise. The present findings therefore contrast recent data reported by Heden et al. (2013) who observed attenuated fasting and postprandial suppression of acylated ghrelin at a

test meal consumed on the morning after exercise completed on the previous evening. The discrepancy in findings is likely related to key differences between protocols such as the time between exercise and blood sampling and the additional bout of exercise performed in the present study. This inconsistency in outcome identifies a need to further scrutinise the protracted impact of exercise on acylated ghrelin.

In the present investigation we saw no change in fasting levels of PYY on day two; however a transient increase in circulating PYY levels was apparent after the second bout of exercise i.e. on day two. This investigation is the first to assess the impact of exercise on the fasting level of PYY on the day after exercise and therefore the lack of change despite a high level of energy expenditure is a novel observation. The short-term increase in PYY after exercise on day two is consistent with previous research which has described a transient increase in PYY in response to moderate-high intensity exercise (Broom, Batterham, King, & Stensel, 2009; Martins, Morgan, Bloom, & Robertson, 2007; Ueda et al., 2009). It is beyond the scope of the present manuscript to speculate on the mechanisms mediating this response; it is clear however that this acute effect is maintained with repeated bouts of exercise on consecutive days. Nonetheless, we did not observe any compensatory down-regulation of PYY in response to exercise-related energy expenditure across the time-frame examined. Furthermore, as shown in previous studies, this exercise-induced change in PYY does not appear to have any influence on appetite or energy intake. PYY has many physiological functions in the body e.g. gastrointestinal transit, substrate metabolism, thermogenesis; and it is possible that the change we are witnessing with exercise is unrelated to appetite control and food intake. Conversely, it is also possible that the response is relevant but is insufficient to overcome ingrained habits and behaviour relating to food intake so that visible effects on these outcomes may only be seen over a greater period of time or with a larger stimulus.

Episodic signals regulating appetite and energy intake function synergistically with additional chronic hormonal mediators which

are primarily sensitive to perturbations in energy balance and bodily energy stores. Within this system leptin and insulin are well defined; being positively associated with energy availability and responding dynamically to perturbations in energy balance (Gautron & Elmquist, 2011; Woods, Lutz, Geary, & Langhans, 2006). Previous research has shown that leptin is sensitive to negative energy balance induced by exercise (≥ 3340 kJ) with compensatory decreases in circulating levels being detectable after a latency period of 12–24 h (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000; Olive & Miller, 2001). In the present investigation, despite a net energy expenditure of ~3765 kJ on day one, we did not detect any change in fasting leptin on the following morning; nor did we see any changes after the second bout of exercise on day two. It is possible that the second bout of exercise on day two may have masked any latent changes which may have otherwise occurred. Furthermore, despite exercise on day one inducing a relative energy deficit of 4234 kJ, the somewhat high amount of energy intake across each trial on day one (control 17268 ± 2775; exercise 16812 ± 2899 kJ) may have been sufficient to block any exercise-related decrease in leptin.

In addition to its central role in regulating glycaemia, insulin is also a tonic signal regulating energy balance either by acting directly within the hypothalamus, or by modulating the brain's sensitivity to episodic appetite signals (McMinn, Sindelar, Havel, & Schwartz, 2000; Woods et al., 2006). Chronically, circulating levels of insulin are positively related to adiposity and insulin resistance within key metabolic tissues. An increase in insulin sensitivity is a well characterised effect of acute exercise that endures for up to 72 h after each individual bout (Hawley & Lessard, 2008). In the present study our participants were young and healthy, with low basal insulin levels. It is therefore perhaps unsurprising that fasting concentrations on day two were not lowered further by prior exercise. On day two we did however observe reduced levels of insulin in response to the morning meal; a response which is consistent with an enhancement of insulin sensitivity induced by exercise. Although important for glycaemic control, this transient response is unlikely to have any influence on energy balance.

This study has some notable limitations. By estimating participants' energy requirements (Mifflin et al., 1990) it appears that participants overate during main trials. This was likely due to the wide selection of foods available at *ad libitum* meals and an associated 'banquet effect.' Consequently, the sensitivity to detect changes in energy intake after consecutive days of exercise may have been reduced. Furthermore, such overconsumption may have also dampened the ability to detect changes in appetite hormones in response to exercise. Another limitation of this study is that performance of a second bout of exercise may have masked any changes in study outcomes on day 2; however this is unlikely given other recent findings of ours (King et al., 2015). Finally, this study focused solely on homeostatic factors contributing to the regulation of appetite and food intake. Recent investigations have highlighted the importance of hedonic factors contributing to these outcomes (Crabtree, Chambers, Hardwick, & Blannin, 2014; Evero, Hackett, Clark, Phelan, & Hagobian, 2012).

In summary, this investigation sought to characterise the impact of two bouts of moderate to high intensity exercise, performed on consecutive mornings, on appetite perceptions, food intake and circulating levels of key appetite regulatory hormones. This study found that besides inducing a transient inhibition of appetite, exercise did not exert any other influence on subjective appetite perceptions and did not alter *ad libitum* energy or macronutrient intake on day one or two. Furthermore, exercise had no impact on circulating levels of leptin or acylated ghrelin, but did augment levels of total PYY and reduced those of insulin, on day two. These findings support previous data in lean individuals, indicating that acute exercise does not induce compensatory appetite or food intake responses within the short-term (King et al., 1994; King, Wasse, & Stensel, 2013; Wasse et al., 2012). This study has demonstrated that this lack of response persists on the day after exercise.

References

Atkinson, G. (2002). Analysis of repeated measurements in physical therapy research. Multiple comparisons amongst level means and multi-factorial designs. *Physical Therapy in Sport*, 3(4), 191–203.

Beaulieu, K., Olver, T. D., Abbott, K. C., & Lemon, P. W. R. (2015). Energy intake over two days is unaffected by acute sprint interval exercise despite increased appetite and energy expenditure. *Applied Physiology, Nutrition, and Metabolism*, 40, 79–86.

Blundell, J. E., & King, N. A. (1998). Effects of exercise on appetite control. Loose coupling between energy expenditure and energy intake. *International Journal of Obesity and Related Metabolic Disorders*, 22, S22–S29.

Borg, G. A. (1973). Perceived exertion. A note on "history" and methods. *Medicine and Science in Sports and Exercise*, 5, 90–93.

Broom, D. R., Batterham, R. L., King, J. A., & Stensel, D. J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology: Regulative Integrative and Comparative Physiology*, 296, R29–R35.

Broom, D. R., Stensel, D. J., Bishop, N. C., Burns, S. F., & Miyashita, M. (2007). Exercise-induced suppression of acylated ghrelin in humans. *Journal of Applied Physiology*, 102, 2165–2171.

Crabtree, D. R., Chambers, E. S., Hardwick, R. M., & Blannin, A. K. (2014). The effects of high-intensity exercise on neural responses to images of food. *The American Journal of Clinical Nutrition*, 99(2), 258–267.

Cummings, D. E., Frayo, R. S., Marmonier, C., Aubert, R., & Chapelot, D. (2004). Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *American Journal of Physiology. Endocrinology and Metabolism*, 287, E297–E304.

De Vriese, C., Perret, J., & Delporte, C. (2010). Focus on the short- and long-term effects of ghrelin on energy homeostasis. *Nutrition (Burbank, Los Angeles County, Calif.)*, 26, 579–584.

Dill, D. B., & Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, 37, 247–248.

Essig, D. A., Alderson, N. L., Ferguson, M. A., Bartoli, W. P., & Durstine, J. L. (2000). Delayed effects of exercise on plasma leptin concentration. *Metabolism: Clinical and Experimental*, 49, 395–399.

Evero, N., Hackett, L. C., Clark, R. D., Phelan, S., & Hagobian, T. A. (2012). Aerobic exercise reduces neuronal responses in food reward brain regions. *Journal of Applied Physiology*, 112(9), 1612–1619.

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity*, 24, 38–48.

Food Standards Agency. (2007). FSA nutrient and food based guidelines for UK institutions. [Online] <http://www.food.gov.uk/sites/default/files/multimedia/pdfs/nutrientinstitution.pdf> Last accessed 15.19.01.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 55, 628–634.

Gautron, L., & Elmquist, J. K. (2011). Sixteen years and counting. An update on leptin in energy balance. *Journal of Clinical Investigation*, 121, 2087–2093.

Hagobian, T. A., Sharoff, C. G., Stephens, B. R., Wade, G. N., Silva, J. E., Chipkin, S. R., et al. (2009). Effects of exercise on energy-regulating hormones and appetite in men and women. *American Journal of Physiology: Regulative Integrative and Comparative Physiology*, 296, R233–R242.

Hawley, J. A., & Lessard, S. J. (2008). Exercise training-induced improvements in insulin action. *Acta Physiologica*, 192, 127–135.

Heden, T. D., Liu, Y., Park, Y., Dellsperger, K. C., & Kanaley, J. A. (2013). Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. *Journal of Applied Physiology*, 115, 680–687.

Hill, B. R., De Souza, M. J., & Williams, N. I. (2011). Characterization of the diurnal rhythm of peptide YY and its association with energy balance parameters in normal-weight premenopausal women. *American Journal of Physiology. Endocrinology and Metabolism*, 301, E409–E415.

Hopkins, M., Gibbons, C., Caudwell, P., Hellstrom, P. M., Näslund, E., King, N. A., et al. (2014). The adaptive metabolic response to exercise-induced weight loss influences both energy expenditure and energy intake. *European Journal of Clinical Nutrition*, 68, 581–586.

Hopkins, M., King, N. A., & Blundell, J. E. (2010). Acute and long-term effects of exercise on appetite control. Is there any benefit for weight control? *Current Opinion in Clinical Nutrition and Metabolic Care*, 13, 635–640.

King, J. A., Garnham, J. O., Jackson, A. P., Kelly, B. M., Xenophontos, S., & Nimmo, M. (2015). Appetite-regulatory hormone responses on the day following a prolonged bout of moderate-intensity exercise. *Physiology and Behaviour*, 141, 23–31.

King, J. A., Wasse, L. K., Ewens, J., Crystallis, K., Emmanuel, J., Batterham, R. L., et al. (2011). Differential acylated ghrelin, Peptide YY3-36, appetite and food intake responses to equivalent energy deficits created by exercise and food restriction. *Journal of Clinical Endocrinology and Metabolism*, 96, 1114–1121.

King, J. A., Wasse, L. K., & Stensel, D. J. (2013). Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite*, 61, 45–51.

King, J. A., Wasse, L. K., Stensel, D. J., & Nimmo, M. A. (2013). Exercise and ghrelin. A narrative overview of research. *Appetite*, *68*, 83–91.

King, N. A., Burley, V. J., & Blundell, J. E. (1994). Exercise-induced suppression of appetite. Effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition*, *48*, 715–724.

King, N. A., Caudwell, P., Hopkins, M., Byrne, N. M., Colley, R., Hills, A. P., et al. (2007). Metabolic and behavioural compensatory responses to exercise interventions. Barriers to weight loss. *Obesity*, *15*, 1373–1383.

King, N. A., Lluch, A., Stubbs, R. J., & Blundell, J. E. (1997). High dose exercise does not increase hunger or energy intake in free living males. *European Journal of Clinical Nutrition*, *51*, 478–483.

Le Roux, C. W., Patterson, M., Vincent, R. P., Hunt, C., Ghatei, M. A., & Bloom, S. R. (2005). Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects. *Journal of Clinical Endocrinology and Metabolism*, *90*, 1068–1071.

Leidy, H. J., Dougherty, K. A., Frye, B. R., Duke, K. M., & Williams, N. I. (2007). Twenty-four hour ghrelin is elevated after calorie restriction and exercise training in non-obese women. *Obesity*, *15*, 446–455.

Mackelvie, K. J., Meneilly, G. S., Elahi, D., Wong, A. C., Barr, S. I., & Chanoine, J. P. (2007). Regulation of appetite in lean and obese adolescents after exercise. Role of acylated and desacyl ghrelin. *Journal of Clinical Endocrinology and Metabolism*, *92*, 648–654.

Manning, S., & Batterham, R. L. (2014). The role of gut hormone peptide YY in energy and glucose homeostasis. Twelve years on. *Annual Review of Physiology*, *76*, 585–608.

Martins, C., Morgan, L. M., Bloom, S. R., & Robertson, M. D. (2007). Effects of exercise on gut peptides, energy intake and appetite. *Journal of Endocrinology*, *193*, 251–258.

McMinn, J. E., Sindelar, D. K., Havel, P. J., & Schwartz, M. W. (2000). Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology*, *141*, 4442–4448.

Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *American Journal of Clinical Nutrition*, *51*(2), 241–247.

Neary, M. T., & Batterham, R. L. (2009). Gut hormones. Implications for the treatment of obesity. *Pharmacological Therapies*, *124*, 44–56.

Olive, J. L., & Miller, G. (2001). Differential effects of maximal and moderate intensity runs on plasma leptin in healthy trained subjects. *Nutrition (Burbank, Los Angeles County, Calif.)*, *17*, 365–369.

Perry, B., & Wang, Y. (2012). Appetite regulation and weight control. The role of gut hormones. *Nutrition and Diabetes*, *2*, e26.

Ross, R., Berentzen, T., Bradshaw, A. J., Janssen, I., Kahn, H. S., Katzmarzyk, P. T., et al. (2008). Does the relationship between waist circumference, morbidity and mortality depend on measurement protocol for waist circumference? *Obesity Reviews*, *9*(4), 312–325.

Schubert, M. M., Desbrow, B., Sabapathy, S., & Leveritt, M. (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite*, *63*, 92–104.

Schubert, M. M., Sabapathy, S., Leveritt, M., & Desbrow, B. (2013). Acute exercise and hormones related to appetite regulation. A meta-analysis. *Sports Medicine*, *44*, 387–403.

Seimon, R. V., Lange, K., Little, T. J., Brennan, I. M., Pilichiewicz, A. N., Feltrin, K. L., et al. (2010). Pooled-data analysis identifies pyloric pressures and plasma cholecystokinin concentrations as major determinants of acute energy intake in healthy, lean men. *American Society for Nutrition*, *92*(1), 61–68.

Stensel, D. (2010). Exercise, appetite and appetite-regulating hormones. Implications for food intake and weight control. *Annals of Nutrition and Metabolism*, *57*, 36–42.

Stubbs, R. J., Sepp, A., Hughes, D. A., Johnstone, A. M., Horgan, G. W., King, N., et al. (2002). The effect of graded levels of exercise on energy intake and balance in free-living men consuming their normal diet. *European Journal of Clinical Nutrition*, *56*, 129–140.

Stubbs, R. J., Sepp, A., Hughes, D. A., Johnstone, A. M., King, N., Horgan, G., et al. (2002). The effect of graded levels of exercise on energy intake and balance in free-living women. *International Journal of Obesity and Related Metabolic Disorders*, *26*, 866–869.

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*, *29*, 71–83.

Tudor-Locke, C., & Bassett, D. R. (2004). How many steps/day are enough? Preliminary pedometer indices for public health. *Sports Medicine*, *34*, 1–8.

Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., & Fujimoto, S. (2009). Changes in gut hormones levels in negative energy balance during aerobic exercise in obese young males. *The Journal of Endocrinology*, *201*, 151–159.

Wasse, L. K., Sunderland, C., King, J. A., Batterham, R. L., & Stensel, D. J. (2012). The influence of rest and exercise at a simulated altitude of 4000 m on appetite, energy intake and plasma concentrations of acylated ghrelin and peptide YY. *Journal of Applied Physiology*, *112*, 552–559.

Whybrow, S., Hughes, D. A., Ritz, P., Johnstone, A. M., Horgan, G. W., King, N., et al. (2008). The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. *British Journal of Nutrition*, *100*, 1109–1115.

Wing, R. R. (1999). Physical activity in the treatment of the adulthood overweight and obesity. Current evidence and research issues. *Medicine and Science in Sports and Exercise*, *31*, s547–s552.

Woods, S. C., Lutz, T. A., Geary, N., & Langhans, W. (2006). Pancreatic signals controlling food intake. Insulin, glucagon and amylin. *Philosophical Transactions of the Royal Society*, *361*, 1219–1235.