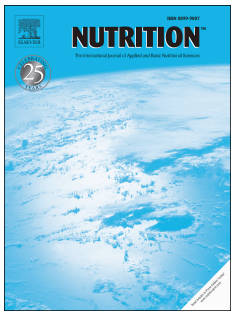
Accepted Manuscript



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PII: S0899-9007(15)00284-1

DOI: 10.1016/j.nut.2015.06.013

Reference: NUT 9562

To appear in: *Nutrition*

Received Date: 9 February 2015

Revised Date: 18 June 2015

Accepted Date: 23 June 2015

Please cite this article as: Clayton DJ, Stensel DJ, James LJ, Effect of breakfast omission on subjective appetite, metabolism, acylated ghrelin and GLP-1_{7-36} during rest and exercise, *Nutrition* (2015), doi: 10.1016/j.nut.2015.06.013.

Effect of breakfast omission on subjective appetite, metabolism, acylated ghrelin and GLP-1_{7-36} during rest and exercise

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Running head: breakfast omission, appetite and energy metabolism

Word count: 4987

Abstract

Breakfast omission induces compensatory eating behaviour at lunch, but often reduces daily energy intake. This study investigated the effect of breakfast omission on within-day subjective appetite, energy expenditure, substrate utilisation and appetite hormone profiles, in response to standardised feeding and exercise. Eight male, habitual breakfast eaters completed two randomised trials. Subjects arrived overnight fasted (0 h), and either consumed (BC) or omitted (BO) a standardised breakfast (Mean (SD) (3085 (217) kJ). Lunch (4162 (510) kJ) and dinner (4914 (345) kJ) were provided at 4.5 and 10 h, respectively and subjects performed 60 min fixed-intensity cycling (50% VO_2peak) at 8 h. Blood samples were collected at 0, 4.5, 6 and 8 h, with expired air and subjective appetite sensations (hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC)) collected throughout. Heart rate and perceived exertion were measured during exercise. Hunger, DTE and PFC were greater and fullness lower during BO (P<0.05) between breakfast and lunch, with no differences after lunch (P>0.193). Resting energy expenditure was greater at 2.5 h during BC (P<0.05) with no other differences between trials (P>0.156). GLP-1_{7-36} was greater (P<0.05) and acylated ghrelin tended to be greater (P=0.078) at 4.5 h during BC. Heart rate was greater on BO (P<0.05) during exercise. The results of this laboratory-controlled study suggest that the effects of breakfast omission are transient and do not extend beyond lunch, even when the negative energy balance created by breakfast omission is sustained via standardised feeding and exercise.

Word Count: 244

Key words: Breakfast skipping, Energy restriction, Energy balance, Meal omission, Energy expenditure.

Introduction

Obesity is the product of prolonged positive energy balance and has been identified as a risk factor for several chronic diseases [1]. Meal omission is a frequently cited method of controlling energy intake [2]. In the absence of behavioural compensation, refraining from eating at a prescribed mealtime, such as breakfast, will create an energy deficit. It is thought that the appetite regulatory system will counter perturbations in energy balance, with metabolic and behavioural compensatory responses that target both energy intake and expenditure [3]. Part of this response may be due to the regulation of appetite hormones such as acylated ghrelin and GLP-$1_{7\text{-}36}$, which have been suggested as biological mechanisms that affect hunger and food intake. Subjective appetite sensations are a valid and reliable method of assessing motivation to eat before and in response to test meals [4], and may also reflect changes in appetite regulatory hormones [5].

Evidence is emerging that energy omitted at breakfast is not fully compensated for over a 24 h period [3,6-8]. Furthermore, it appears that any compensatory eating behaviour is exhibited during the next meal [3,6], and it is currently unclear whether the increased energy intake at this meal suppresses further intake throughout the day, or whether the appetitive effects of breakfast omission are diminished after the initial stimulation of food intake.

Energy expenditure may also be altered in response to fluxes in energy balance due to breakfast omission. In one study energy expenditure was shown to decrease in the morning in response to breakfast omission, but was not different over a 24 h period [10]. In this study, energy intake at lunch and dinner was increased to account for the energy omitted at breakfast, whereas complete compensation rarely occurs in response to acute breakfast omission [11]. Low intensity physical activity has been shown to reduce in response to chronic breakfast omission [8]. An exercise intervention may have the potential to offset this decrement somewhat, provided the subjective response to exercise and/or adherence is not

affected by breakfast omission. Lifestyle interventions that combine both dietary restriction and exercise have been shown to be more effective for weight management in the long-term [12]; therefore it is important to consider the effect that a given dietary intervention has on physical activity.

A more complete understanding of the hormonal and metabolic responses to breakfast omission is warranted. This this study was designed to investigate the appetite and metabolic responses to breakfast omission, with energy intake at lunch and dinner held constant, which has not been previously investigated. Therefore, the aim of this study was to investigate the effect of breakfast omission on subjective appetite sensations and metabolism in response to standardised feeding and sub-maximal exercise.

Methods

Subjects

Eight healthy, recreationally active males (age: 27 (6) y; weight: 75 (8.1) kg; height: 1.74 (0.07) m; BMI: 25 (2) $kg \cdot m^{-2}$; body fat: 18 (3) %; VO_2peak: 53.4 (5.1) $mL \cdot kg^{-1}$ (mean (SD)) volunteered to participate in the study. All subjects were regular breakfast eaters, reported to have been weight stable for 6 months, and were not restrained, disinhibited or hungry eaters [13]. The study was approved by the Loughborough University Ethics Approvals (Human Participants) Sub-committee, and all subjects provided full written consent and completed a health screen questionnaire prior to participation.

Preliminary trial

Subjects' height (Seca, Birmingham, UK), weight (Adam AFW-120K, Milton Keynes, UK) and body fat percentage [14] were determined. Cycling VO_2peak was determined using a discontinuous incremental exercise test (Lode Corival, Groningen, Holland). Increments lasted 4 min, were separated by ~5 min rest, and work load was increased until volitional exhaustion. Expired air was collected into a Douglas bag during the final min of each stage, with heart rate (Polar Beat, Kemple, Finland) and rating of perceived exertion (RPE) [15] recorded at the end of each increment.

Pre-trial standardisation

Dietary intake and physical activity in the 48 h preceding the first experimental trial were recorded by each subject in a diary and these patterns were replicated in the 48 h before the next trial. Subjects also abstained from alcohol and strenuous exercise during this period.

Protocol

Subjects completed two experimental trials; breakfast consumption (BC) and breakfast omission (BO). Trials were separated by at least 7 days, conducted at the same time of day, on the same day of the week and administered in a randomised, counterbalanced order.

Subjects travelled to the laboratory via motorised transport arriving at approximately 08:00, following at least a 10 h fast, and were weighed nude. After 30 min supine rest (0 h), blood and expired air samples were collected. Subjective appetite sensations were then assessed on a visual analogue scale (VAS) before subjects consumed either a standardised breakfast (BC) or no breakfast (BO). Subjects then rested quietly in the laboratory. At 4.5 h, a blood sample was collected, before a standardised lunch was consumed. Subjects again rested in the laboratory with blood samples collected at 6 h and 8 h. Subjects then completed 60 min cycling at 50% VO_2peak (8-9 h). Heart rate and RPE were recorded after 20, 40 and 60 min of exercise. One hour after exercise (10 h) a standardised dinner meal was consumed. Subjects then left the laboratory, but were not permitted to eat until the following morning, completing VAS scales at 12, 13.5 and 24 h..

Standardised meals

During BC subjects were provided a standardised breakfast of 25% estimated daily energy requirements (DER), determined by multiplying resting metabolic rate (RMR) [16] by a physical activity level of 1.7. Breakfast consisted of crisped rice cereal, semi-skimmed milk, white bread, butter, strawberry jam and orange juice (Tesco, Cheshunt, UK). During BO, subjects ingested water (624 (44) mL) to match water contained in the breakfast of BC. Subjects were provided the same lunch and dinner on both trials. Lunch consisted of ham sandwiches, crisps and yoghurt (35% DER) and dinner consisted of pasta, tomato sauce, cheese and olive oil (40% DER). Subjects consumed each meal gradually over a 30 min period (Table 1).

After breakfast, subjects ingested 45 $mL \cdot kg^{-1}$ body mass water on each trial (2318 (284) mL). This water was distributed so that 100 mL was provided every 20 min during exercise. Of the remaining water, 25% was ingested at lunch and dinner, and 12.5 % at 2.5, 7, 12 and 13.5 h.

Subjective appetite sensations

Hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) were assessed on 100 mm VAS at 0, 0.5, 1.5, 2.5, 3.5, 4.5, 5, 6, 7, 8, 9, 10, 10.5, 12, 13.5, 24 h. Verbal anchors of 'not at all/no desire at all/none at all' and 'extremely/a lot' were placed at 0 and 100 mm, respectively.

Expired air samples

Ten min expired air samples were collected at 0, 2.5, 4.5, 6, 8 and 10 h in a supine position after 20 min supine rest [17]. The first 5 min was discarded and the second 5 min was collected into a Douglas bag. O_2 and CO_2 concentration were determined using a paramagnetic oxygen analyser and an infra-red carbon dioxide analyser, respectively (1400 Series, Servomex, East Sussex, UK). These were calibrated prior to each sample using certified reference gases (BOC, Guildford, UK). The volume (Harvard Dry Gas Meter, Harvard Ltd, Kent, UK) and temperature (Edale thermister, Cambridge, UK) of each expired air sample were also determined. Energy expenditure and substrate oxidation were calculated using the stoichiometric equations described by Frayn [18]. Four min expired air samples were collected after 20, 40 and 60 min of exercise, of which the first 2 min of each sample was discarded.

Blood sampling and analysis

Blood samples (12 mL) were drawn after 30 min supine rest, at 0, 4.5, 6 and 8 h via venepuncture of an antecubital vein. Five mL blood was immediately mixed with 50 µL Dipeptidyl-peptidase 4 inhibitor (DPP4-010, Merck Millipore, Watford, UK) and dispensed into an EDTA tube (1.75 $mg \cdot mL^{-1}$), for determination of active glucagon-like peptide-1 ($GLP\text{-}1_{7\text{-}36}$) by ELISA (EGLP-35K, Merck Millipore, Watford, UK). Two and a half mL blood was dispensed into an EDTA tube containing 10 $\mu L \cdot mL^{-1}$ of a potassium phosphate buffer (PBS) (0.05 M), *P*-hydroxymercuribenzonic acid (PHMB) (0.05 M) and sodium hydroxide solution (NaOH) (0.006 M) for determination of acylated ghrelin concentration by

ELISA (A05106, Bioquote Ltd, York, UK). Two and a half mL of blood was dispensed into an EDTA tube for measurement of blood glucose concentration (GOD-PAP method, Randox Laboratories Ltd, Crumlin, UK) and insulin concentration by ELISA (DX-EIA-2935, Immunodiagnostic Systems, Boldon, UK).

All samples were centrifuged at 1750*g* for a total of 15 min in a refrigerated centrifuge (4°C). After 10 min of centrifugation, the supernatant (1 mL) of the PHMB/PBS/NaOH treated blood was combined with 100 $\mu L \cdot mL^{-1}$ HCl (1 M) before all samples were centrifuged for a further 5 min. The supernatant of each sample was then removed and stored at -20°C until frozen and then transferred to -80°C for later analysis.

A further 2 mL blood was collected into an EDTA tube and used for the determination of haemoglobin (via the cyanmethaemoglobin method) and haematocrit (via micro-centrifugation), and used to estimate changes in plasma volume relative to baseline [19].

Statistical Analysis

Data was analysed using SPSS 21.0 (SPSS Inc., Somers, NY, USA). Area under the curve (AUC) values were calculated using the trapezoidal method and averaged over time. Subjective appetite sensations were then separated into three periods (0-4.5 h, 5-10 h, 10.5-24 h) and energy expenditure was presented as total (0-10 h), and also divided into two time periods (0-4.5 h, 5-10 h). Correction of hormone concentrations for plasma volume change did not alter the results so the unadjusted values are presented. All data were checked for normality of distribution using a Shapiro-Wilk test. Data containing one factor were analysed using a t-test or Wilcoxon signed-rank test, as appropriate. Data containing two factors were analysed using a two-way repeated measures ANOVA, followed by *post-hoc* Bonferroni-adjusted paired t-tests or Bonferroni-adjusted Wilcoxon signed-ranks, as appropriate. Data sets were determined to be significantly different when $P<0.05$. Data are presented as mean (SD) unless otherwise stated.

Results

Pre-trial values

Pre-trial body mass (P=0.155), subjective appetite sensations (all P>0.346), RMR (P=0.393), carbohydrate oxidation (P=0.815) and fat oxidation (P=0.290) were not different between trials. Plasma concentrations of glucose (P=0.512), insulin (P=0.488), acylated ghrelin (P=0.526) and GLP-$1_{7\text{-}36}$ (P=0.636) were also not different between trials at baseline.

Subjective appetite sensations

All subjective appetite sensations showed an interaction effect (P<0.001). Sensations of fullness were lower concurrent with greater hunger, DTE (all P<0.01) and a tendency for greater PFC (P=0.078) at 0.5 h during BO compared to BC. Between 1.5 and 3.5 h, lower fullness and greater hunger, DTE and PFC (all P<0.05) was observed during BO compared to BC. Lower hunger (P<0.01), PFC (P<0.05) and a tendency for lower DTE (P=0.078) was found immediately prior to lunch (4.5 h) during BC compared to BO, but there was no difference between trials for fullness (P=0.234). After lunch there were no differences between trials for any appetite variables (5.5-24 h) (P>0.125; Fig 1).

Data was divided into 3 sections for AUC analysis; baseline to lunch (0-4.5 h), post-lunch to dinner (5-10 h) and post-dinner (10.5-24 h). These analyses revealed differences between trials for all appetite variables between baseline and lunch (all P<0.05), with no further differences between trials (all P>0.719; Fig 1).

Energy expenditure and substrate oxidation

Due to a fault with the gas collection equipment during one trial for one subject, this subjects air samples were removed from the analysis. Therefore data from 7 subjects is presented.

Respiratory exchange ratio (RER) showed an interaction effect (P<0.05) and was greater at 2.5 (P<0.01), 4.5 (P<0.05) and 10 h (P<0.05) during BC compared to BO (Fig 2a).

Carbohydrate oxidation was greater at 2.5 (P<0.001) and 4.5 h (P<0.05) during BC, but fat oxidation was not different between trials (P=0.413).

There was an interaction effect for energy expenditure (P<0.01), with greater energy expenditure at 2.5 h during BC (P<0.05) compared to BO, with no other differences between trials (P>0.156; Fig 2b). AUC analyses revealed a tendency for increased energy expenditure at 0-4.5 h (P=0.066) during BC, but no difference at 5-10 h (P=0.523) or in total (P=0.193).

Blood parameters

Plasma acylated ghrelin concentrations showed a main effect of time (P<0.001), but no interaction effect (P=0.238). Bloxplot analysis revealed one consistently outlying subject within the data set, exhibiting acylated ghrelin concentrations ~11 standard deviations greater than the mean of the 7 other subjects. Therefore, this subject was removed from the analysis. After removal, an interaction effect was identified (P<0.05). Acylated ghrelin tended to be higher during BC compared to BO at 4.5 h (P=0.078). Compared to 0 h, acylated ghrelin was greater at 4.5 h during BC (P<0.05) and reduced at 6 h during BO (P<0.05) (Table 2).

An interaction effect (P<0.05) was identified for GLP-$1_{7\text{-}36}$, with greater concentrations at 4.5 h during BC compared to BO (P<0.05). Compared to baseline, GLP-$1_{7\text{-}36}$ was greater at 6 and 8 h during BC and at 8 h during BO (P<0.05; Table 2)

Plasma insulin showed a main effect of time (P<0.001) and was greater than baseline at 6 h during BC (P<0.05) as well as at 6 and 8 h during BO (P<0.05). No interaction effect was observed for plasma insulin (P=0.468) or glucose (P=0.067) concentration (Table 2).

Exercise responses

There was a main effect of trial for heart rate (P<0.05), which was elevated at 60 min during BO compared to BC (P<0.05), and tended to be elevated at 40 min (P=0.068). VO_2 (P=0.503), RER (P=0.135), carbohydrate oxidation (P=0.143), fat oxidation (P=0.143), energy expenditure (P=0.289) and RPE (P=0.129) were similar between trials (Table 3).

Discussion

This investigation found that subjective appetite sensations, appetite hormones and energy expenditure were not different after lunch, regardless of whether the subject consumed or omitted breakfast. Therefore, it appears that the appetitive and metabolic effects of breakfast omission are transient and might be offset by a standardised lunch. Breakfast omission also does not influence perception of effort or energy expenditure during 60 min of steady-state cycling exercise performed 3 h after lunch. This data suggests that occasional breakfast omission may not stimulate appetite and promote energy intake as has been previously inferred (20).

Regularity of breakfast consumption has been identified as a risk factor for obesity, with correlational evidence to suggest that habitual breakfast consumers have a lower BMI than breakfast omitters [20]. However, habitual breakfast consumers also tend to exhibit healthy lifestyle practices, such as greater levels of physical activity [21] and improved dietary profiles [22] than breakfast omitters, making causal mechanisms difficult to elucidate. Acute studies that have directly manipulated the consumption or omission of breakfast have generally reported that the omission of breakfast will increase appetite and induce compensatory eating behaviour at lunch [6,9]. Whilst one study found that the energy omitted at breakfast was fully compensated for at an *ad-libitum* lunch meal [23], the majority of studies have reported that energy intake at a single meal [6,9,24] or over a 24 h period [3,6-8] is rarely sufficient to fully compensate for the energy omitted at breakfast. In the current investigation, the energy consumed at each meal was fixed so an increase in energy intake could not occur. These results demonstrate that even when energy consumed at lunch is controlled, there were no differences in appetite sensations or concentrations of acylated ghrelin and GLP-$1_{(7\text{-}36)}$ after lunch.

The transient suppression of appetite after consumption compared to omission of breakfast has previously been observed after an *ad-libitum* lunch meal, which was used to gauge voluntary food intake [6,9]. However, the present investigation has demonstrated that appetite in the post-lunch period can be offset by an absolute energetic load, as opposed to subjects eating to satiation. This effect was shown to persist throughout the rest of the day, despite subjects consuming ~3000 kJ less during BO. Therefore, controlling food intake at subsequent meals does not appear to affect the appetitive response to acute breakfast omission, and this could allow greater energy deficits to be achieved, compared to when *ad-libitum* meals are consumed. However, it should be noted that subjective appetite sensations do not always accurately predict subsequent food intake [25].

Energy expenditure increased at 2.5 h during BC, compared to BO. This would be anticipated due to dietary induced thermogenesis (DIT). The thermogenesis associated with feeding is dependent on the energetic load and the macronutrient content of the meal. When the breakfast meal was broken down into its constituents, the estimated DIT of the meal was approximately 9.8% of the total energy content of the meal, which is in line with the typically reported DIT of a mixed meal of 10% [26]. Taking this into account, it is likely that the majority of the post-prandial increase in energy expenditure at 2.5 h was due to an increase in DIT. Even including DIT in the morning, AUC analysis did not reveal any differences between trials over the 10 h expired air sampling period. This is in line with the finding of Kobayashi *et al.* [10] who reported that breakfast consumption increased energy expenditure in the morning, compared to breakfast omission, but 24 h energy expenditure was not different between trials. In this study, the energy content of the lunch and dinner meals were increased in the no breakfast condition to match total daily energy intake between trials. The results of the current study have therefore extended those of Kobayashi *et al.* [10] and

277 determined that, even in an energy deficient state, energy expenditure is not affected by
278 occasional breakfast omission.
279 The nature of measuring energy expenditure in a laboratory requires the subject to be at rest,
280 and therefore changes in habitual activity patterns may have been overlooked. Betts *et al.* [8]
281 found that over a 6 week period, breakfast omission decreased habitual energy expenditure
282 by ~1850 $kJ \cdot d^{-1}$ compared to when breakfast was consumed. This was attributed to a decrease
283 in low intensity physical activity, as opposed to a reduction in exercise intensity/duration,
284 which was not measured in the current investigation. It is possible that physical activity of
285 this nature is subconsciously affected by breakfast omission. Results of the present study
286 show that any reduction in energy expenditure is not due to changes in resting metabolism,
287 and therefore the incorporation of exercise into daily routines may help offset this reduction
288 in low intensity physical activity, provided adherence to exercise is not affected by the
289 dietary intervention.
290 Time constraints of a working lifestyle often restrict time to exercise to the morning or
291 evening, with evening exercise classes associated with increased alertness and enthusiasm, as
292 well as being deemed to require less effort than morning classes [27]. This may help improve
293 adherence to an exercise program in the long term. The current study implemented a
294 prescribed exercise protocol on both experimental trials, and found that heart rate was
295 elevated during exercise on BO compared to BC. This suggests that subjects were more
296 physiologically challenged during exercise on BO, although this was not reflected in RPE,
297 VO_2 or energy expenditure. Digestion and absorption of nutrients from the gut is a process
298 that requires oxygen to be delivered to the splanchnic tissue, typically achieved via a
299 redistribution of blood away from the skeletal muscle or an increase in cardiac output [28].
300 During exercise, where the skeletal muscle requirements for oxygen are high, an increase in
301 heart rate would facilitate meeting the metabolic requirements of skeletal muscle activity and

digestion and absorption of nutrients. Heart rate may have been increased to a greater extent during exercise on BO, as splanchnic blood supply for digestion and absorption of nutrients may be prioritised, due to the subjects peripheral fuel supply being reduced during BO compared to BC [29]. Noradrenaline is an indicator of peripheral sympathetic nervous activity, and has been shown to peak after breakfast, and progressively decline following lunch and dinner meals [30]. By removing breakfast during BO, it is possible that the peak sympathetic response occurred after lunch, which subsequently increased heart rate to a greater extent during exercise on BO.

The increase in appetite over the morning period during BO has been suggested to lead to the consumption of energy dense snacks [31], and indeed an increase in snacking behaviour has been observed in a previous study [3]. Elevated levels of the appetite stimulating hormone ghrelin and suppression of satiety hormones, such as GLP-1, have been suggested as biological mechanisms that stimulate hunger and promote food intake [5,32]. In the present study, GLP-$1_{7\text{-}36}$ was suppressed immediately prior to lunch in BO compared to BC, which may be linked to greater fullness and lower hunger, DTE and PFC in the present study, following breakfast consumption. Interestingly, acylated ghrelin tended to be higher prior to lunch during BC compared to BO (P=0.078). The reason for this is unclear; however ghrelin has been shown to respond diurnally, peaking at anticipated meal times. Extending the overnight fast during BO may have affected this diurnal variation, which may be governed primarily by post-prandial decreases rather than pre-prandial increases [33]. After lunch, there were no differences in acylated ghrelin and GLP-$1_{(7\text{-}36)}$ suggesting, in line with the subjective appetite sensations, there was no additional desire to increase food intake after lunch.

In conclusion, this laboratory-controlled investigation found that subjective appetite sensations, acylated ghrelin, GLP-$1_{(7\text{-}36)}$ and resting energy expenditure were not different,

independent of whether breakfast was consumed or omitted. This was found in spite of sustaining the negative energy balance induced by breakfast omission, via standardised lunch and dinner feeding and a prescribed exercise protocol. Consuming breakfast in the morning appears to only transiently suppress appetite compared to when breakfast is omitted, and appetite can be offset with provision of a standardised lunch meal. This extends findings from *ad-libitum* feeding studies, and suggests that a similar effect can be achieved with a standardised lunch meal, which may help enhance the energy deficit that can be achieved. Therefore, this study supports occasional breakfast omission as a means to reduce daily energy intake.

Acknowledgements

This 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. Study conception, design, data collection and analysis were conducted by DJC and LJJ. The manuscript was written by DJC, with assistance from LJJ and DJS. All authors approved the final manuscript.

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434 **Captions (Figures 1 & 2)**

435

436 **Figure 1.** Subjective feelings of hunger (A), fullness (B), desire to eat (C) and prospective
437 food consumption (D) (left panel) and AUC analysis (right panel) during BC (■) and BO (□).
438 Data are mean (SE) for the left panel and mean (SD) right panel. White rectangle indicates
439 breakfast, hatched rectangles indicate standard meals, black rectangle represents exercise. †
440 Significantly different to BC (P<0.05).

441

442 **Figure 2.** Respiratory exchange ratio (RER) during BC (■) and BO (□) (A); and Resting
443 energy expenditure (B). Data are mean (SD). On x-axis, white rectangle indicates breakfast,
444 hatched rectangle indicates standard meal, black rectangle represents exercise. † Significantly
445 different to BC (P<0.05); * Significantly different to baseline (P<0.05).

446

447 **Tables with captions**

448 **Table 1.** Energy and macronutrient intake. Values are mean (SD).

	CHO (g)	PRO (g)	FAT (g)	FIBRE (g)	ENERGY (kJ)
			Breakfast		
BC	130.0 (9.1)	19.5 (1.4)	13.7 (1.0)	4.5 (0.3)	3085 (217)
BO	0	0	0	0	0
			Lunch		
BC	118.9 (8.3)	38.6 (2.7)	41.1 (2.9)	12.0 (0.8)	4162 (301)
BO					
			Dinner		
BC	150.6 (10.5)	41.2 (2.9)	43.2 (3.0)	6.8 (0.5)	4914 (345)
BO					
			Total		
BC	399.6 (28.0)	99.4 (7.0)	94.4 (13.0)	23.2 (1.6)	12162 (988)
BO	270.0 (18.9)	79.9 (5.6)	80.7 (12.3)	18.8 (1.3)	9077 (789)

449

450

451 **Table 2.** Plasma concentrations of acylated ghrelin, GLP-$1_{7\text{-}36}$, insulin and glucose. Data are

452 mean (SD). † Significantly different to BC; * Significantly different to baseline (P<0.05).

	0 h	4.5 h	6 h	8 h
		Acylated Ghrelin (pg·mL^{-1})		
BC	162 (132)	213 (147)*	114 (132)	156 (150)
BO	168 (150)	178 (171)	111 (148)*	150 (165)
		GLP-$1_{7\text{-}36}$ (pM)		
BC	9.67 (8.49)	10.13 (8.22)	12.34 (7.67)*	11.72 (8.32)*
BO	9.92 (9.78)	8.52 (8.83)†	13.01 (7.92)	12.85 (8.88)*
		Insulin (µlU·mL^{-1})		
BC	9.56 (4.29)	7.03 (3.98)	30.09 (11.68)*	18.49 (8.67)
BO	8.74 (3.90)	7.56 (3.35)	34.90 (15.86)*	15.58 (3.78)*
		Glucose (mmol·L^{-1})		
BC	5.33 (0.22)	4.77 (0.42)	5.28 (0.79)	5.17 (0.45)
BO	5.35 (0.23)	5.26 (0.47)	5.69 (0.88)	4.88 (0.56)

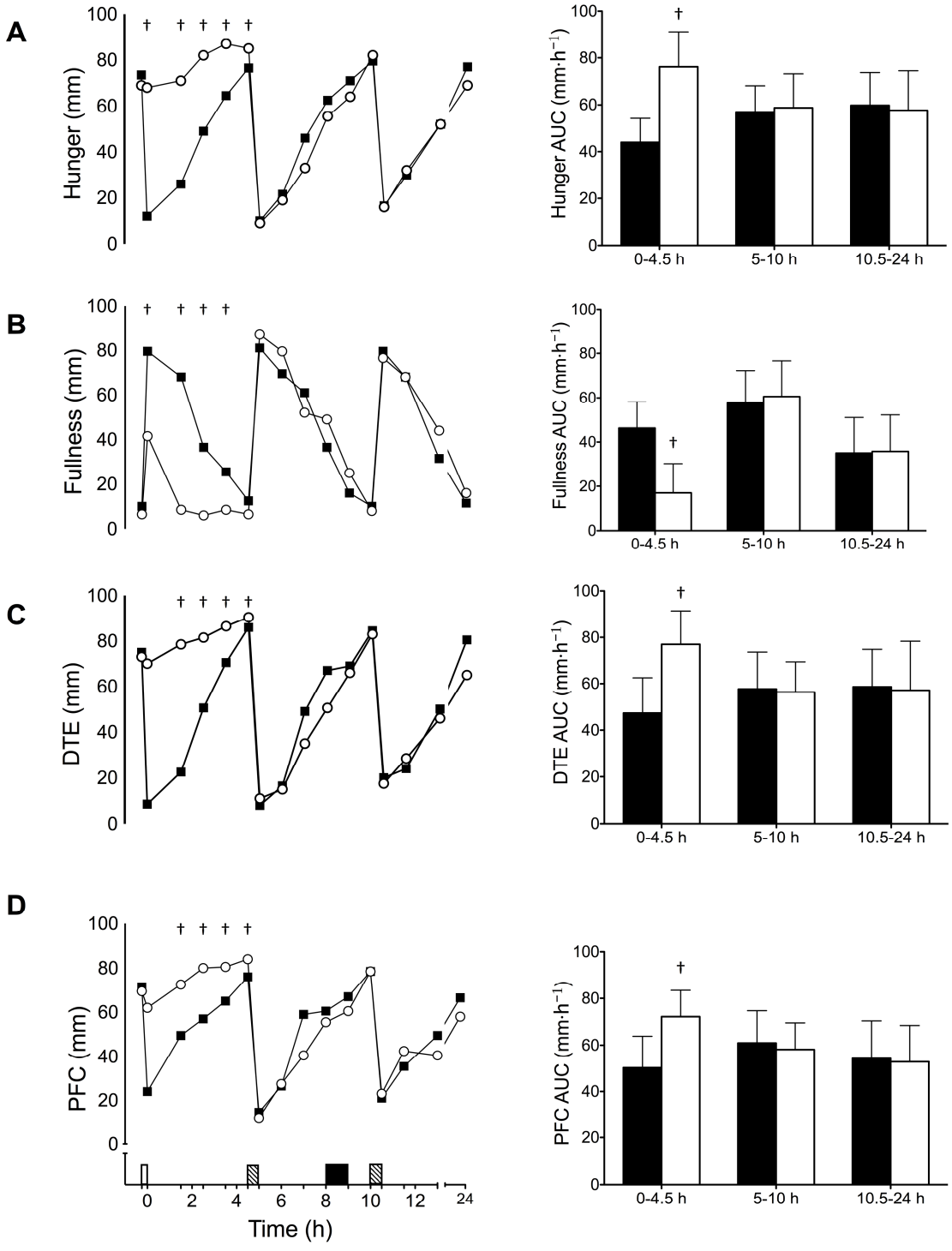
453

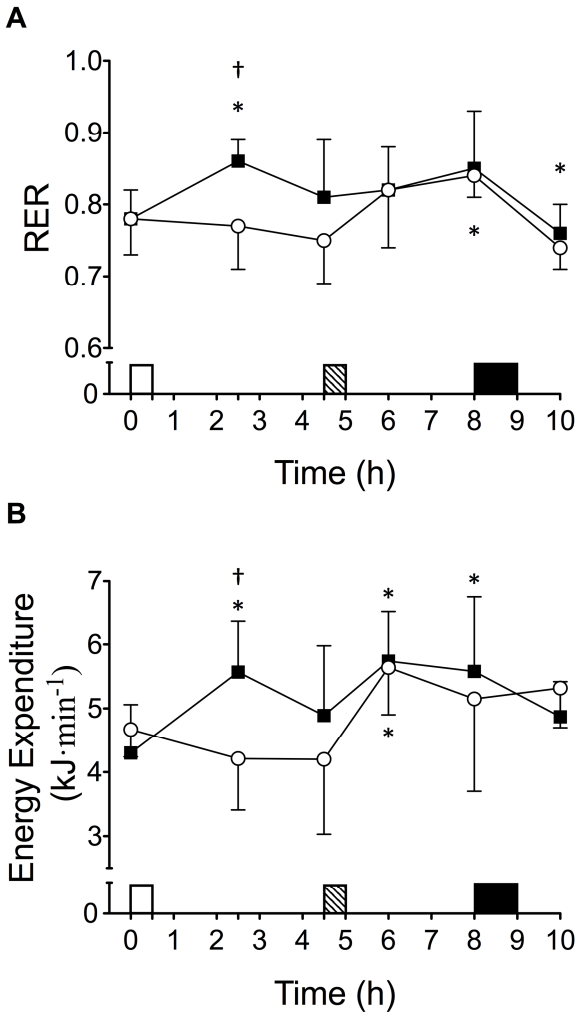
454

455 **Table 3.** Variables collected during exercise. Data are mean (SD). † Significantly different to
456 BC (P<0.05).

	BC	**BO**	***P*-value**
VO_2 ($L \cdot min^{-1}$)	1.95 (0.25)	1.92 (0.26)	0.503
RER	0.92 (0.03)	0.90 (0.01)	0.107
Carbohydrate oxidation ($g \cdot min^{-1}$)	1.93 (0.34)	1.72 (0.14)	0.143
Fat oxidation ($g \cdot min^{-1}$)	0.25 (0.14)	0.31 (0.08)	0.143
Energy Expenditure ($kJ \cdot min^{-1}$)	42.05 (5.01)	40.78 (5.16)	0.289
Heart rate ($beats \cdot min^{-1}$)	130 (5)	134 (6)†	0.032
RPE	11 (1)	12 (1)	0.129

457


A
Hunger (mm)
Hunger AUC (mm·h⁻¹)
B
Fullness (mm)
Fullness AUC (mm·h⁻¹)
C
DTE (mm)
DTE AUC (mm·h⁻¹)
D
PFC (mm)
PFC AUC (mm·h⁻¹)
0-4.5 h
5-10 h
10.5-24 h
Time (h)



Highlights

- Appetite responses to breakfast omission/ consumption were compared
- Lunch and dinner intake were standardised
- Subjective appetite was not different between trials after lunch
- GLP-$1_{7\text{-}36}$ and acylated ghrelin were not different between trials after lunch
- The effects of breakfast omission appear transient and do not extend beyond lunch