Effect of 24-h severe energy restriction on appetite regulation and ad libitum energy intake in lean men and women

Effect of 24 h severe energy restriction on appetite regulation and *ad-libitum* energy intake in lean males and females

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Abbreviations:

ANOVA – analysis of variance

AUC – area under the curve

CO_2 – carbon dioxide

DTE – desire to eat

EB – energy balance trial

EDTA – ethylenediaminetetraacetic acid

EER – estimated energy requirements

ELISA – enzyme-linked immunosorbent assay

ER – energy restriction trial

GLP-$1_{7\text{-}36}$ – active glucagon-like peptide-1

NEFA – non-esterified fatty-acids

O_2 – oxygen

PFC – prospective food consumption

SD – standard deviation

SER – severe energy restriction

Abstract

Background: Intermittent severe energy restriction (SER) can induce significant weight loss, but the appetite regulatory response to SER is unknown and may dictate long-term dietary adherence and acceptability.

Objective: Determine the effect of 24 h SER on appetite regulation, metabolism and energy intake.

Design: Eighteen lean males and females completed two three-day trials, in randomized counterbalanced order. On day 1 subjects consumed standardized diets containing 100% (9.3 (1.3) MJ; EB) or 25% (2.3 (0.3) MJ; ER) of estimated energy requirements. On day 2, a standardized breakfast was consumed (2.5 (0.3) MJ), with plasma concentrations of acylated ghrelin, glucagon-like peptide-1 (GLP-$1_{7\text{-}36}$), insulin, glucose and non-esterified fatty-acids (NEFA) determined for 4 h. *Ad-libitum* energy intake was assessed at lunch and dinner, with subjective appetite and resting metabolism assessed throughout. On day 3, *ad-libitum* energy intake was assessed at breakfast and via weighed food records.

Results: Energy intake was 7% greater on day 2 ($P<0.05$) during ER, but not significantly different on day 3 ($P=0.557$). Subjective appetite was greater during ER on day 1 ($P<0.0001$) and during the morning of day 2 ($P<0.05$), but was not significantly different after lunch ($P>0.145$). Postprandial acylated ghrelin concentration was lower during ER ($P<0.05$), whilst postprandial GLP-$1_{7\text{-}36}$ concentration was not significantly different between trials ($P=0.784$). Postprandial glucose ($P<0.05$) and NEFA ($P<0.0001$) concentrations were greater during ER, whilst insulin concentration tended to be greater ($P=0.06$). Energy expenditure was lower during ER in the morning ($P<0.01$), but was not significantly different after lunch ($P=0.665$).

Conclusions: In lean young adults, 24 h severe energy restriction transiently elevated subjective appetite and marginally increased energy intake, but hormonal appetite markers did

not respond in a manner indicative of hyperphagia. These results suggest intermittent SER might be useful to attenuate energy intake and control body weight in this population.

Key words: appetite hormones; energy balance; calorie restriction; intermittent fasting; alternate day fasting; weight management; dieting.

Introduction

Obesity is a major risk factor for several chronic diseases, and represents a considerable health and economic burden worldwide (1-2), emphasizing a need for the development of achievable weight management strategies. Whilst the majority of weight management research tends to focus of methods to assist obese individuals lose weight, recent research suggests that part of this problem is attributable to lean individuals gaining weight throughout adulthood, eventually contributing to increasing rates of obesity (3). An improved understanding of how weight loss strategies translate to weight maintenance strategies will help to curtail the prevalence of obesity in the future.

Traditional weight management diets involve daily energy restriction to induce a moderate energy deficit over time (4). This method of energy restriction is successful for ~30% of dieters, but the requirement for daily adherence to the diet may compromise long-term adherence to the diet (5). Recently, intermittent severe energy restriction has been proposed as an alternative to daily energy restriction (6). This involves severely restricting energy intake intermittently (1-4 days a week), with adequate (7-8) or *ad-libitum* (9-11) energy intake on other days. Under tightly controlled experimental conditions, weight loss of 4-12% has been reported after 8-24 weeks (7-11), which is comparable with weight loss reported from daily energy restriction diets (6).

Studying the acute effects of severe energy restriction may elucidate some of the mechanisms of action. Persistent hunger is often cited as a reason for poor adherence to weight management regimes (12), suggesting that long-term adherence and weight loss may depend on how that dietary intervention influences appetite. Ghrelin and glucagon-like peptide-1 (GLP-1) are gut hormones that may influence appetite to correct perturbations in energy balance (13-14). However, little is known about how appetite hormone profiles respond after

53 short periods of severe energy restriction. A recent study reported that 48 h of severe energy
54 restriction produced a postprandial appetite hormone profile that would be expected to
55 suppress, rather than stimulate appetite, in male and female soldiers (15), but the large
56 exercise component and incorporation of meal replacement gels possibly limits the translation
57 of these findings to weight management settings.

58 The aim of this study was to examine the effect of 24 h of severe energy restriction (providing
59 25% of estimated energy requirements) on subjective and hormonal appetite regulation, as
60 well as *ad-libitum* food intake, compared to an adequate energy control diet. We hypothesized
61 that, relative to the control trial, acylated ghrelin response would be greater and GLP-1_{3-36}
62 reduced after 24 h severe energy restriction, and that this would be concurrent with
63 upregulated subjective appetite and increased *ad-libitum* energy intake.

Methods

Subjects

Data collection took place between October 2013 and June 2015 in the nutrition laboratories at Loughborough University, UK. After institutional ethical approval, ten healthy males and eight healthy females (Table 1) provided written consent and completed the study. Subjects were not restrained, disinhibited or hungry eaters (16), had been weight stable for >6 months and were not currently dieting. Female participants completed a menstrual cycle questionnaire, and were tested during the post-menstruation follicular phase (~5-12 days after start of menstruation). Sample size was estimated from energy intake data from a similar study (17), data from our laboratory using similar *ad-libitum* meals (18) and an estimated between group correlation of 0.5 (G*Power 3.1.6; Dusseldorf, Germany). Using an α of 0.05 and statistical power of 0.95, it was determined at least 16 subjects would be required to reject the null hypothesis.

Study design

During a 1-day preliminary trial, height, weight and body fat percentage (19) were determined and subjects were familiarized with the *ad-libitum* meals and blood sampling procedures. Subjects then completed two 3-day experimental trials, administered in a crossover, randomized, counterbalanced order. Trials were separated by ≥14 days for males and exactly 1 menstrual cycle for females. On day 1 of each experimental trial, subjects received either 100% (energy balance; EB) or 25% (energy restriction; ER) of their estimated energy requirements (EER). On day 2 and 3, food intake, behavior and metabolic responses to each diet were assessed (**Figure 1**). The primary outcome measures were energy intake, subjective appetite and appetite hormone responses (acylated ghrelin and GLP-$1_{7\text{-}36}$). The secondary

outcome measures were glucose, insulin, non-esterified fatty-acids (NEFA) and expired gas measures.

Pre-trial standardization

Alcohol consumption and strenuous exercise were not permitted in the 2 days before, or during the 3-day experimental trials. Subjects recorded all dietary intake and any habitual physical activity during the 2 day prior to the first experimental trial and replicated these patterns in the 2 day prior to the second experimental trial.

Protocol

For each trial, subjects arrived at the laboratory via motorized transport at ~07:30 on three consecutive mornings, after a ≥10 h overnight fast and after voiding, nude body mass was measured (Adam Equipment Co, Milton Keynes, UK). On day 1, expired gas and blood (via venepuncture) samples were collected and subjective appetite assessed (~08:00; -24 h). Subjects left the laboratory at ~08:30, after receiving all food and drink for the day, along with instructions on when to consume each item. On day 2, an indwelling cannula was inserted, and the measurements from day 1 were repeated (~08:00; 0 h). A standardized breakfast consisting of cereal, semi-skimmed milk, white bread, butter and jam (2.5 (0.3) MJ; 16 (2) g protein; 93 (13) g carbohydrate; 16 (2) g fat; 3 (0) g fiber) and providing 25% EER was then consumed over 20 min. Subjects then rested in the laboratory, with subjective appetite sensations, blood and expired gas collected periodically between breakfast and lunch. The cannula was removed after the final collection, and an *ad-libitum* multi-item lunch was provided (~12:00-12:30; 4-4.5 h). After lunch, subjects rested in the laboratory, with further expired gas (5, 7, 9, 11 h) and subjective appetite sensations collected (5, 6, 7, 8, 8.25, 9, 10, 11 h). A standardized yoghurt and cereal bar snack (0.9 (0.1) MJ; 4 (1) g protein; 25 (3) g carbohydrate; 10 (1) g fat; 1 (0) g fiber) was consumed at ~16:00 (8 h), and an *ad-libitum*

dinner was provided at ~19:00-19:30 (11-11.5 h), with subjective appetite assessed immediately after dinner (11.5 h). On day 3, blood (via venepuncture) and expired gas samples were collected, subjective appetite assessed (~08:00; 24 h), and an *ad-libitum* porridge breakfast was provided 24-24.5 h. Final subjective appetite sensations were collected at 24.5 h, and subjects completed a weighed record of all food and drink consumed for the remainder of the day (24.5-48 h).

Standardized diet preparation

Diets were tailored to individual preferences and formulated to contain palatable and recognizable foods to ensure adherence. Estimated resting metabolic rate (20) was multiplied by a sedentary physical activity level of 1.4 to determine EER for each subject. During EB, 100% of EER was provided (**Table 2**), distributed into 4 meals; breakfast (20%; 08:00), lunch (30%; 12:00), afternoon snack (10%; 16:00) and dinner (40%; 19:00). During ER, 25% of EER was provided (Table 2), divided between lunch (34%; 12:00) and dinner (66%; 19:00), with a water-only breakfast (0%; 08:00) provided isovolume to the water content of the breakfast provided in EB. Additional water intake was prescribed at 35 $mL \cdot kg^{-1}$ body mass (2438 (347) mL) and was evenly distributed throughout the day. Similar foods were provided on day 1 during both trials. Because of the beneficial effects of dietary protein on preservation of fat-free mass and increasing satiety (21), the diet provided on day 1 of the ER trial was created by removing or reducing high carbohydrate and high fat foods from the EB diet (i.e. bread, pasta, mayonnaise and snack foods).

Energy intake

Energy intake was assessed at a multi-item *ad-libitum* lunch (4-4.5 h), a homogenous *ad-libitum* dinner (11-11.5 h), a homogenous *ad-libitum* breakfast (24-24.5 h) and via habitual food records (24.5-48 h). *Ad-libitum* meals provided in the laboratory were served in an

isolated feeding booth, as described previously (18). The multi-item lunch consisted of bread, cooked meats, butter, mayonnaise, fruit, salad, biscuits and crisps; the homogenous dinner consisted of pasta, tomato sauce and olive oil (6.27 (0.11) $kJ \cdot g^{-1}$; 12, 68, 18 and 2 % of energy provided by protein, carbohydrate, fat and fiber, respectively); and the homogenous breakfast consisted of porridge oats and semi-skimmed milk (4.40 (0.05) $kJ \cdot g^{-1}$; 17, 59, 22 and 2 % of energy provided by protein, carbohydrate, fat and fiber, respectively). At *ad-libitum* meals, subjects were explicitly instructed to eat until they were 'comfortably full and satisfied' and the amount consumed at each meal was quantified by weighing food items before and after the meal, with macronutrient and energy intake ascertained from manufacturer values. Food records were analyzed from manufacture values where possible or using NetWisp dietary analysis software (Netwisp Inc., Chicago, USA).

Energy expenditure and substrate oxidation

After 20 min of supine rest, 10 min expired gas samples were collected in accordance with the guidelines described by Compher (22). The first 5 min of each collection was discarded, with the second 5 min collected and analyzed for O_2 and CO_2 concentration (1400 series, Servomex, East Sussex, UK), volume (Harvard Dry Gas Meter, Harvard Ltd, Kent, UK) and temperature (Edale thermistor, Cambridge, UK). Energy expenditure and substrate oxidation were calculated from these values (23).

Subjective appetite

Hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) were assessed pre-breakfast (-24 h), post-breakfast (-23.5 h), pre-lunch (-20 h), post-lunch (-19.5 h), pre-dinner (-13 h) and post-dinner (-12.5 h) on day 1; pre-breakfast (0 h), post-breakfast (0:20 h) and at 1, 2, 3, 4, 4.5, 5, 6, 7, 8, 8.25, 9, 10, 11, 11.5 h on day 2; and pre-breakfast (24 h) and post-breakfast (24.5 h) on day 3. Ratings were provided on 100 mm visual analogue scales

159 with verbal anchors ‘not at all/ none at all/ no desire at all’ and ‘extremely/ a lot’ placed at 0
160 and 100 mm, respectively.

161 *Blood sampling and analysis*

162 Blood samples (15 mL) were drawn from an antecubital vein after 30 min of supine rest.
163 Blood was dispensed into tubes containing EDTA (1.75 $mg \cdot mL^{-1}$) pre-treated for the
164 determination of acylated ghrelin and active glucagon-like peptide-1 ($GLP\text{-}1_{7\text{-}36}$)
165 concentrations, as previously described (24), and plasma was separated by centrifugation (15
166 min; 1750 *g*; 4°C). Concentrations of $GLP\text{-}1_{7\text{-}36}$, (CV: 4.8%; Merck Millipore, Watford, UK),
167 acylated ghrelin (CV: 3.7%; Bioquote Ltd, York, UK), and insulin (CV: 3.2%; Immuno-
168 diagnostics systems, Boldon, UK) were determined by ELISA. The limit of detection for each
169 variable was determined by the lowest standard provided in the ELISA kit, and this value was
170 assigned to any measured concentration below this value, as per manufacturer instructions.
171 Glucose (CV: 0.5%; Horiba, Northampton, UK) and non-esterified fatty acid (CV: 2.9%;
172 NEFA; Randox Laboratories Ltd, Crumlin, UK) concentrations were determined by
173 colorimetric assay using a benchtop analyzer (Pentra 400, Horiba, Northampton, UK). Two
174 mL of whole blood was used for determination of haemoglobin (via the cyanmethaemoglobin
175 method) and haematocrit (via microcentrifugation), and used to estimate changes in plasma
176 volume relative to baseline (25).

177 *Statistical analysis*

178 Data were analyzed using SPSS 21.0 (Somers, NY, USA). Due to problems with blood
179 sampling, blood samples were only collected for 16 (8 male; 8 female) of the 18 subjects. For
180 all other measures n=18. Using the change in plasma volume to correct blood variables did
181 not alter the results, so the unadjusted values are presented. All data were checked for
182 normality using a Shapiro-Wilk test. Data containing two factors were analyzed using a two-

way repeated measures ANOVA, followed by *post-hoc* paired t-tests or Wilcoxon signed rank tests, as appropriate. The Holm-Bonferroni adjustment was used to control the family-wise error rate. Total area under the curve (AUC) values were calculated using the trapezoidal method and were analyzed using a t-test or Wilcoxon signed-rank test, as appropriate. AUC for blood parameters was calculated in response to the standard breakfast (0-4 h). AUC for subjective appetite sensations were calculated for day 1 (-24-0 h), in response to the standard breakfast (0-4 h), during the afternoon (4.5-11 h) and during the evening/ overnight (11.5-24 h) on day 2. AUC for energy expenditure and substrate oxidation were calculated in response to the standard breakfast (0-4 h) and during the afternoon (4.5-11 h). Additionally, gender was entered as a between-subjects factor in repeated measures ANOVA to test for gender-by-trial-by-time interactions, and gender-by-trial interactions (AUC and energy intake). Data sets were determined to be significantly different when $P<0.05$. Data are presented as mean (SD) unless otherwise stated.

Results

Gender analysis

There were main effects of gender for some variables, with plasma NEFA concentration greater in females ($P<0.05$), and *ad-libitum* energy intake ($P<0.001$), energy expenditure ($P<0.001$), carbohydrate oxidation ($P<0.001$) and body mass ($P<0.01$) greater in males. There were no gender-by-trial interaction effects for energy intake at any *ad-libitum* meal ($P>0.338$) or reported energy intake on day 3 ($P=0.469$). There was a gender-by-trial interaction effect for fullness AUC between lunch and dinner on day 2 ($P<0.05$), with fullness lower in males on ER compared to EB ($P<0.05$). There were no other gender-by-trial ($P<0.274$) or gender-by-trial-by-time ($P<0.342$) interaction effects for AUC or raw data, respectively. Therefore, male and female data are presented together.

Energy intake

On day 2, *ad-libitum* energy intake was greater at lunch (EB: 4.3 (1.5) MJ; ER: 4.8 (1.3) MJ; $P<0.05$) and tended to be greater at dinner (EB: 4.3 (0.1) MJ; ER: 4.6 (1.2) MJ; $P=0.056$) during ER. Therefore, total *ad-libitum* energy intake on day 2 was 7% greater during ER compared to EB ($P<0.05$). On day 3, *ad-libitum* energy intake was not significantly different at breakfast (EB: 2.2 (0.6) MJ; ER: 2.4 (0.5) MJ; $P=0.162$) and there was no difference in reported energy intake over the remainder of the day (EB: 9.0 (3.0) MJ; ER: 8.5 (2.8) MJ; $P=0.362$). Over the 2 day period, the increase in energy intake (0.5 (2.9) MJ) was only sufficient to replace ~7% of the energy deficit created on day 1. Therefore energy intake over the 3-day trial was 6.5 (3.3) MJ greater during EB ($P<0.00001$; Table 2).

Energy expenditure and substrate oxidation

There was a main effect of time ($P<0.0001$), but no trial ($P=0.153$) or interaction (P=0.101) effects for energy expenditure (**Figure 2**). Post-breakfast energy expenditure AUC was lower during ER ($P<0.01$) but was not significantly different between trials after lunch ($P=0.665$; Figure 2) or at 24 h ($P=0.867$; data not shown). For carbohydrate and fat oxidation, there were time ($P<0.00001$), trial ($P<0.001$) and interaction ($P<0.001$) effects (Figure 2). Carbohydrate oxidation was lower between 0-4 h ($P<0.05$) and fat oxidation greater at 0, 1, 3 and 4 h ($P<0.05$) during ER compared to EB. Post-breakfast AUC was lower for carbohydrate oxidation ($P<0.00001$) and greater for fat oxidation ($P<0.0001$; Figure 2) during ER. Furthermore, post-lunch AUC was greater for fat oxidation ($P<0.05$) and lower for carbohydrate oxidation ($P<0.05$; Figure 2) during ER.

Blood parameters

There were time ($P<0.00001$), trial ($P<0.05$) and interaction ($P<0.00001$) effects for plasma glucose concentration (**Figure 3**). Plasma glucose was lower at 0 h and greater between 1-1.5

h (P<0.05) during ER. Plasma glucose AUC was greater during ER compared to EB (P<0.05). For plasma insulin concentration, there was a main effect of time (P<0.0001), but no main effect of trial (P=0.057) or interaction effect (P=0.120; Figure 3). Plasma insulin AUC tended to be greater during ER (P=0.06). There were time (P<0.00001), trial (P<0.0001) and interaction (P<0.00001) effects for plasma NEFA concentration (Figure 3). Plasma NEFA concentration was greater between 0-1 h (P<0.01) and tended to be greater at 1.5 h (P=0.076) during ER. Plasma NEFA AUC was also greater during ER (P<0.0001). There were time (P<0.00001), trial (P<0.05) and interaction (P<0.01) effects for plasma acylated ghrelin concentration (**Figure 4**). Acylated ghrelin concentration was greater at 0 and 3 h during EB compared to ER (P<0.05) and acylated ghrelin AUC was greater during EB (P<0.05). There was a main effect of time (P<0.001), but no trial (P=0.513) or interaction (P=0.568) effect for plasma GLP-$1_{7\text{-}36}$, and plasma GLP-$1_{7\text{-}36}$ AUC was not significantly different between trials (P=0.528; Figure 4).

Subjective appetite sensations

AUC for hunger, DTE and PFC were greater, and fullness lower for Day 1 (P<0.00001) and post-breakfast on day 2 (P<0.05). There were no differences in post-lunch (P>0.145) or overnight (P>0.214) AUC for appetite sensations (**Figure 5**).

Body mass

Morning body mass on day 1, 2 and 3, respectively was 69.2 (9.4) kg, 68.9 (9.3) kg and 68.8 (9.4) kg during EB and 69.5 (9.5) kg, 68.4 (9.2) kg and 68.9 (9.4) kg during ER. There were time (P<0.001) and interaction (P<0.001) effects for body mass. Body mass loss from day 1 to day 2 was greater during ER compared to EB (P<0.001) and body mass on day 2 was lower during ER compared to EB (P<0.001). Day 3 body mass was not significantly different between trials (P=0.594).

Discussion

The aim of the current study was to compare the effects of 24 h of adequate (100% EER consumed) or severely restricted (25% EER consumed) energy intake on appetite regulation and *ad-libitum* energy intake in the subsequent 48 h. The main findings were that 24 h of severe energy restriction caused a transient elevation in subjective appetite and increased *ad-libitum* energy intake by ~7% in the first 24 h and by ~2% overall. In addition there was no difference in subjective appetite between trials after an *ad-libitum* lunch and 24 h of severe energy restriction did not promote an appetite hormone response indicative of hyperphagia. These results suggest that short periods of severe energy restriction may reduce energy intake, and assist with appetite control in lean males and females.

Previous studies have reported that lean individuals do not accurately adjust energy intake in response to a dietary induced energy deficit (15,17,26,27). Consistent with the current study, either no compensation (26) or only partial compensation (15,17,27) in the 1-4 days after an acute (24-48 h) period of severe or complete energy restriction has been reported. Consequently, the majority of the energy deficit induced by energy restriction in these studies was preserved. *Ad-libitum* energy intake was ~7% greater during ER on day 2, with no difference in energy intake on day 3, and average energy intake over the 3-day study was ~20% (2.1 MJ) lower during ER compared to EB. Therefore, short-term severe energy restriction appears to represent a viable strategy for attenuating energy intake in lean males and females.

Subjects reported greater hunger, DTE, PFC and lower fullness on day 1 during ER compared to EB. Johnstone *et al.* (17) similarly reported elevated subjective appetite after 36 h of complete energy restriction, but after consumption of an *ad-libitum* breakfast, subjective appetite was comparable to an energy balance control trial. In the current study, subjective appetite remained elevated throughout the morning during ER after a standardized breakfast

containing 25% EER. This suggests that the breakfast used in the current study was not sufficient to offset appetite to the same extent as the *ad-libitum* breakfast provided by Johnstone *et al.* (17). However, subjective appetite sensations were not significantly different between trials after the *ad-libitum* lunch. This suggests subjective appetite can be offset by an *ad-libitum* meal independent of energetic compensation, and thereafter maintenance of the energy deficit might be achieved in the absence of elevated subjective appetite.

Acylated ghrelin is an orexigenic hormone that has been suggested to initiate food intake as concentrations increase before and decrease after eating (28). Therefore, acylated ghrelin might be expected to increase after energy restriction, as a mechanism to restore energy balance homeostasis (13). However, 1-4 days of energy restriction of varying severity has shown no effect on fasting and/or postprandial ghrelin concentrations (29-31). The current study differs further from the anticipated response of acylated ghrelin to an energy deficit, finding a reduction in fasting and postprandial acylated ghrelin concentrations after 24 h of severe energy restriction. Whilst counter-intuitive, these findings are consistent with a recent study reporting suppressed postprandial acylated ghrelin concentrations after consumption of a diet providing 10% EER for 2-days and including a large component of physical exercise (15). Intralipid infusion has previously been show to suppress acylated ghrelin (32), so the elevated plasma NEFA concentrations observed in the current study during ER, may explain why acylated ghrelin was suppressed in this, as well as a previous (15) study.

Intravenous infusion of the anorexigenic hormone GLP-$1_{7\text{-}36}$ has been shown to suppress appetite and food intake, suggesting a role in meal termination and post-meal satiety (14). Whilst GLP-$1_{7\text{-}36}$ concentration has been shown to decrease after weight loss (33-34), 24 h severe energy restriction did not affect fasting or postprandial GLP-$1_{7\text{-}36}$ concentrations in the current study, suggesting this might not be an important regulator of short-term energy balance. GLP-$1_{7\text{-}36}$ is also an incretin hormone which responds to ingested nutrients in the

stomach and stimulates insulin secretion prior to nutrient absorption (35). As no between-trial differences in insulin concentration were observed, it appears that neither the anorexigenic or insulinotropic actions of GLP-1_{7-36} were affected by 24 h of severe energy restriction in the current study. However, GLP-1_{7-36} is rapidly degraded into its inactive form (GLP-1_{9-36}) by the enzyme dipeptidyl peptidase IV upon release from intestinal L-cells (36). Therefore, GLP-1_{7-36} could potentially still influence appetite centrally without being detected peripherally.

Whilst dietary interventions are generally developed to aid weight loss in overweight and obese individuals, research suggests that BMI progressively increases throughout adulthood (4). To prevent the progression towards obesity, effective methods to assist weight management in lean individuals might be as important as weight loss in overweight/ obese individuals. Intermittent severe energy restriction has been shown to effectively reduce weight under tightly controlled conditions (7-11) and therefore could also be a successful strategy of reducing energy intake for weight maintenance. However, compliance to periods of very-low energy intake under free-living conditions has not been fully elucidated. Persistent hunger and requirements for daily adherence have been highlighted as reasons for poor compliance to diets (5,12), and could ultimately dictate long-term success. In the current study, the appetite hormone response to severe energy restriction was not indicative of elevated appetite, but paradoxically, subjective appetite was increased and energy intake was ~12% greater at lunch. This may question the role of these hormones in the short-term regulation of energy balance, and may also reveal the complexity of human eating behavior, which is likely governed by hedonic factors, in addition to physiological cues. However, subjective appetite was offset after lunch and there was no further difference in energy intake. Therefore a flexible dietary approach permitting *ad-libitum* eating with intermittent periods of very-low energy intake may assist with appetite control and aid long-term dietary compliance.

A small (~0.2 $kJ \cdot min^{-1}$), transient reduction in resting energy expenditure was observed during ER, but ER and EB were not significantly different over the assessment period. Whilst this minor decrement is unlikely to influence energy balance, the laboratory procedures utilized are likely to have restricted physical activity, preventing a comprehensive energy expenditure assessment in this study. An increase in fat and reduction in carbohydrate oxidation was observed on day 2 during ER. This has been reported previously (37-39), and is indicative of altered nutrient supply and/ or endogenous stores. Carbohydrate provision in the current study may have been insufficient to meet obligate glucose requirements (40), resulting in an increase in lipolysis to provide NEFA for energy metabolism to preserve endogenous glycogen (40).

Glucose AUC was greater and insulin AUC tended to be greater (P=0.06) on ER, suggesting glycaemic control was impaired after 24 h severe energy restriction. This has been observed after short periods of complete energy restriction (41), and could be driven by elevated plasma NEFA concentrations, which may reduce the rate of glucose uptake into the muscle (42-43). However, the practical relevance of this finding is unclear and has not been determined after chronic intermittent severe energy restriction. Fasting insulin sensitivity has been shown to improve after 4 months of intermittent (2 days per week) severe energy restriction, but the effect of long term severe energy restriction and refeeding cycles on postprandial insulin sensitivity is unknown and warrants further investigation.

In conclusion, 24 h of severe energy restriction causes a transient increase in subjective appetite and a small increase in energy intake during the subsequent 24 h. Hormonal markers of appetite were not upregulated after severe energy restriction, and did not respond in a manner indicative of hyperphagia. Therefore, an acute period of severe energy restriction may assist with energy balance management in lean males and females. Future studies should aim to examine the chronic effects of intermittent severe energy restriction on appetite regulation.

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Tables

Table 1. Baseline subject characteristics[1]

	Males (n= 10)	**Females (n = 8)**
Age (y)	24 (2)	22 (2)
Weight (kg)	74. 4 (7.2)	63.8 (8.6)
Height (m)	1.78 (0.06)	1.61 (0.05)
BMI ($kg \cdot m^{-2}$)	24 (2)	24 (2)
Body fat (%)	14 (4)	27 (5)

[1]Data are means (standard deviations)

Table 2. Energy intake and macronutrient intake during each day of the experimental trial[1]

	Day 1		Day 2		Day 3		Daily averaged intake	
	EB	**ER**	**EB**	**ER**	**EB**	**ER**	**EB**	**ER**
Protein (g)	97 (14)	60 (9) †	95 (21)	99 (20)	117 (43)	115 (45)	103 (22)	91 (21) †
Carbohydrate (g)	294 (41)	56 (8) †	403 (89)	424 (100)	336 (96)	316 (98)	344 (67)	265 (56) †
Fat (g)	70 (9)	9 (1) †	90 (22)	100 (21) †	90 (36)	90 (31)	83 (19)	66 (12) †
Fibre (g)	11 (2)	3 (1) †	22 (5)	23 (6)	26 (7)	27 (10)	20 (4)	18 (5) †
Energy (MJ)	9.3 (1.3)	2.3 (0.3) †	12.0 (2.4)	12.8 (2.5) †	11.2 (3.0)	10.9 (2.9)	10.8 (2.1)	8.7 (1.6) †

[1] n = 18. Data are mean (SD)

EB, energy balance trial; ER, energy restricted trial. † indicates ER trial was significantly different from EB (P<0.05) as determined by paired t-test.

Figure Legends

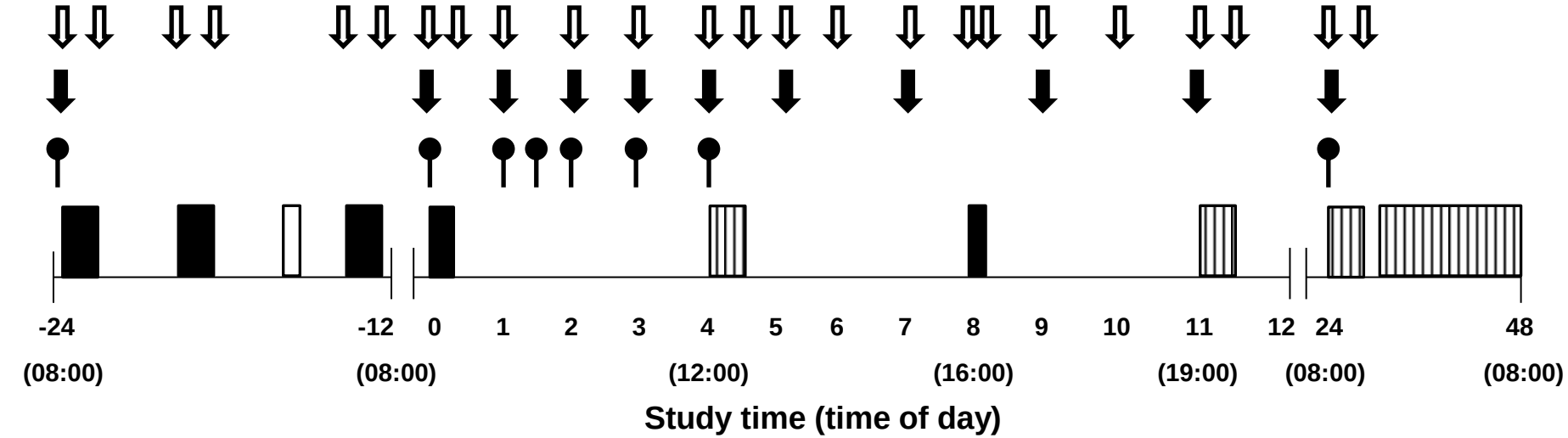
Figure 1. Schematic representation of study protocol. EB, energy balance trial; ER, energy restriction trial.

Figure 2. Energy expenditure (A) and substrate oxidation (B) on Day 2 of the experimental trial, during energy balance trial (EB; ■) and energy restriction trial (ER; ○). Data points are means with vertical error bars representing standard deviation (n=18). Bar charts represent mean energy expenditure (C) and substrate oxidation (D) area under the curve during EB (■) and ER (□), with vertical error bars representing standard deviation. There was a main effect of time (P<0.0001), but no trial (P=0.153) or interaction (P=0.101) effects for energy expenditure, and there were main time (P<0.00001), trial (P<0.001) and interaction (P<0.001) effects for carbohydrate and fat oxidation, examined by two-way repeated measures ANOVA. † indicates where ER values were significantly different from EB, determined by Bonferroni-Holm adjusted paired t-test (P<0.05).

Figure 3. Plasma glucose (A), insulin (B), and non-esterified fatty-acids (NEFA) (C) during energy balance trial (EB; ■) and energy restriction trial (ER; ○). Data points are means with vertical error bars representing standard deviation (n=16). Bar charts represent mean area under the curve response (0-4 h) to a 2.5 (0.3) MJ standardized breakfast during EB (■) and ER (□), with vertical error bars representing standard deviation. There were main effects of time for plasma glucose, insulin and NEFA (all P<0.0001), a main effect of trial for plasma glucose and NEFA (both P<0.05) but not insulin (P=0.057), and interaction effects for plasma glucose and NEFA (both P<0.00001), but not insulin (P=0.120), examined by two-way repeated measures ANOVA. † indicates where ER values were significantly different from EB, determined by Bonferroni-Holm adjusted paired t-test (P<0.05).

Figure 4. Plasma acylated ghrelin (A) and glucagon-like peptide-1 (GLP-1_{7-36}) (B) during energy balance trial (EB; ■) and energy restriction trial (ER; ○). Data points are means with vertical error bars representing standard deviation (n=16). Bar charts represent the mean area under the curve response (0-4 h) to a (2.5 (0.3) MJ) standardized breakfast during EB (■) and ER (□), with vertical error bars representing standard deviation. There were main effects of time for acylated ghrelin and GLP-1_{7-36} (both $P<0.01$), a main effect of trial for acylated ghrelin ($P<0.05$), but not GLP-1_{7-36} ($P=0.513$), and an interaction effect for acylated ghrelin ($P<0.01$), but not GLP-1_{7-36} ($P=0.568$), examined by two-way repeated measures ANOVA. † indicates where ER values were significantly different from EB, determined by Bonferroni-Holm adjusted paired t-test ($P<0.05$).

Figure 5. Area under the curve for hunger (A), fullness (B), desire to eat (DTE) (C), and prospective food consumption (PFC) (D), on Day 1, and during the morning (0-4 h), afternoon (5-11 h), and evening (11.5-24 h) of Day 2, during energy balance trial (EB; ■) and energy restriction trial (ER; □). Bars are mean values with vertical error bars representing standard deviation (n=18). † indicates values were significantly different from EB determined Bonferroni-Holm adjusted paired t-test ($P<0.05$).



Standardised meal provided on EB and ER

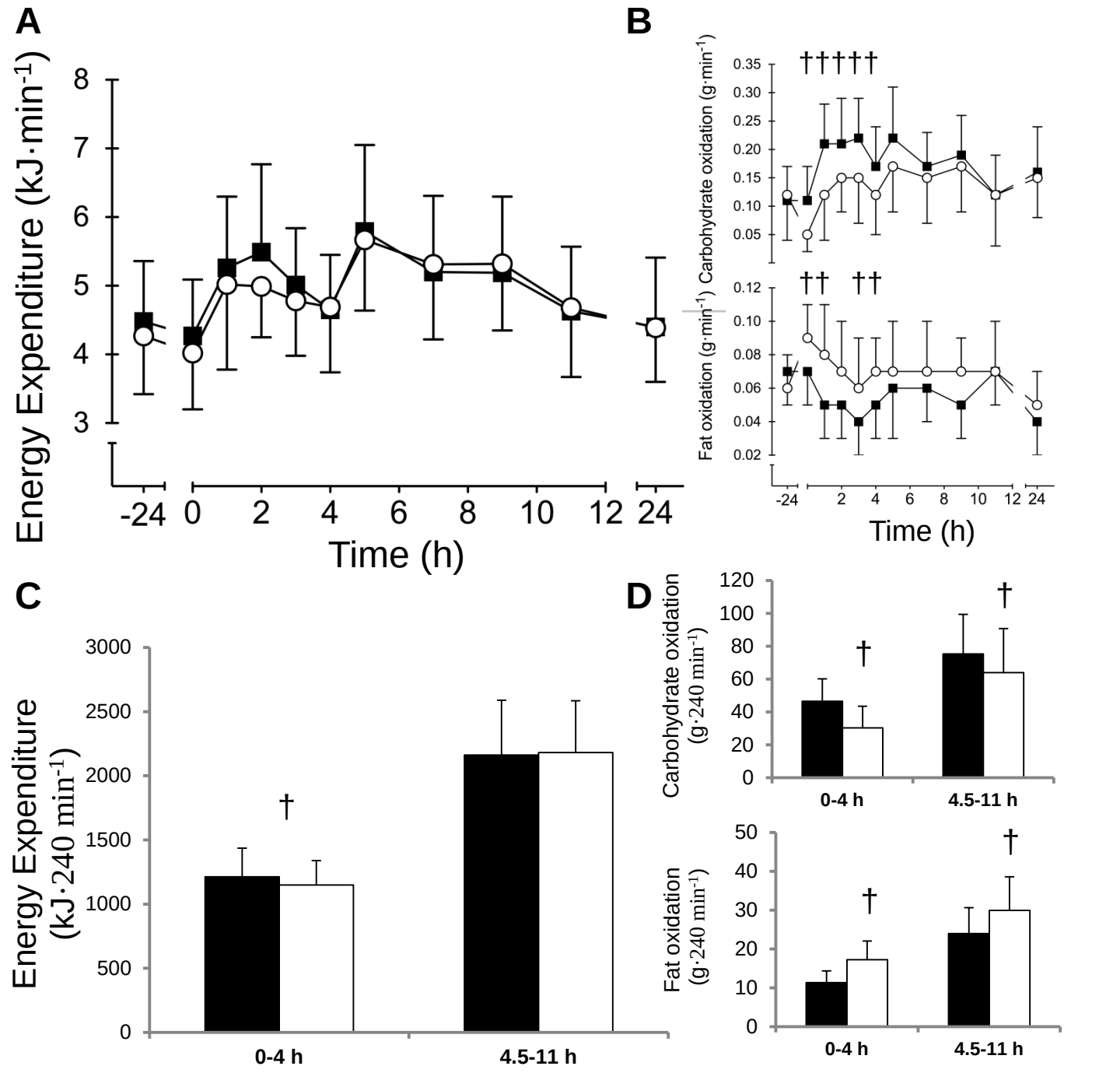
Standardised meal provided on EB only

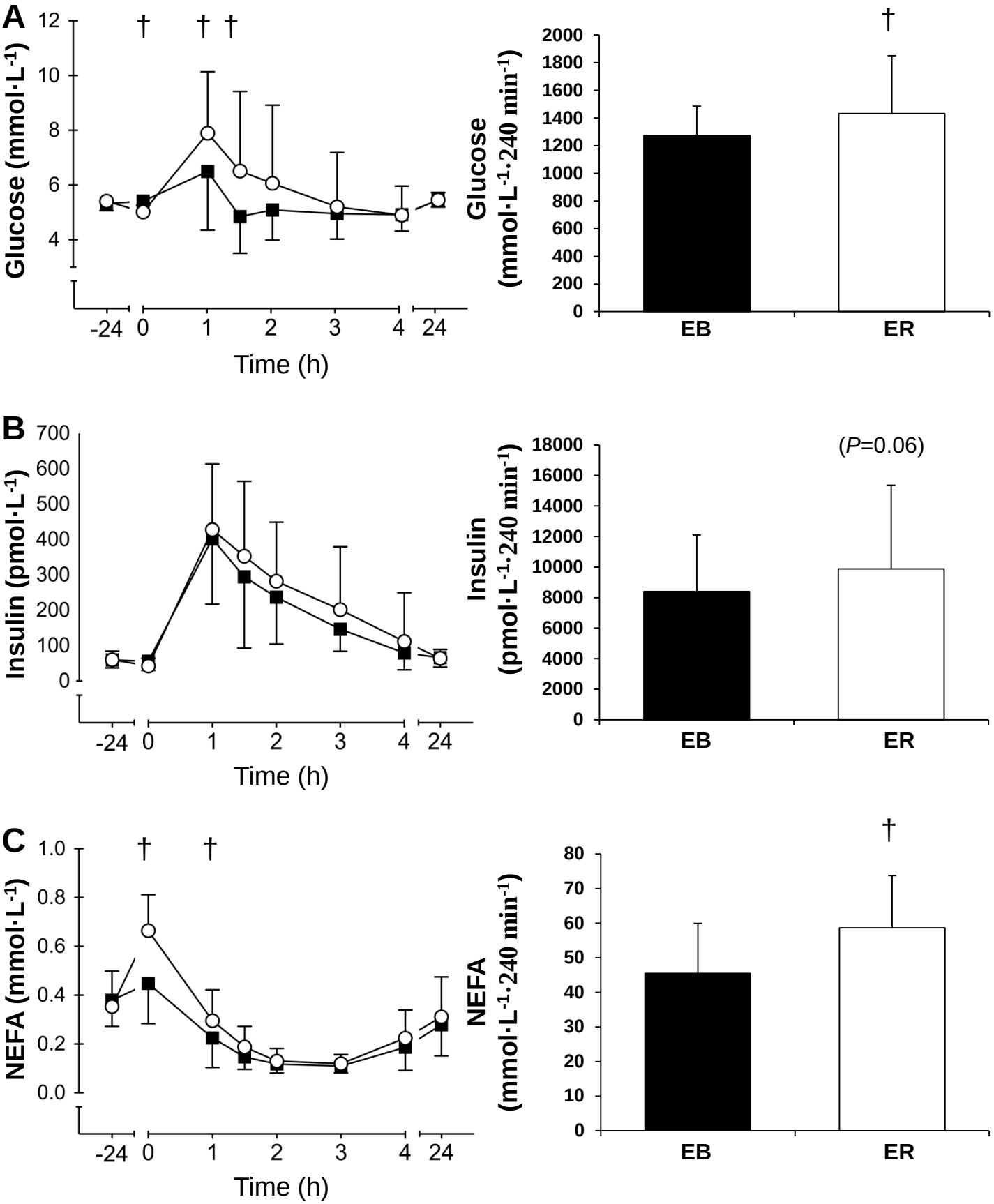
Ad-libitum feeding period

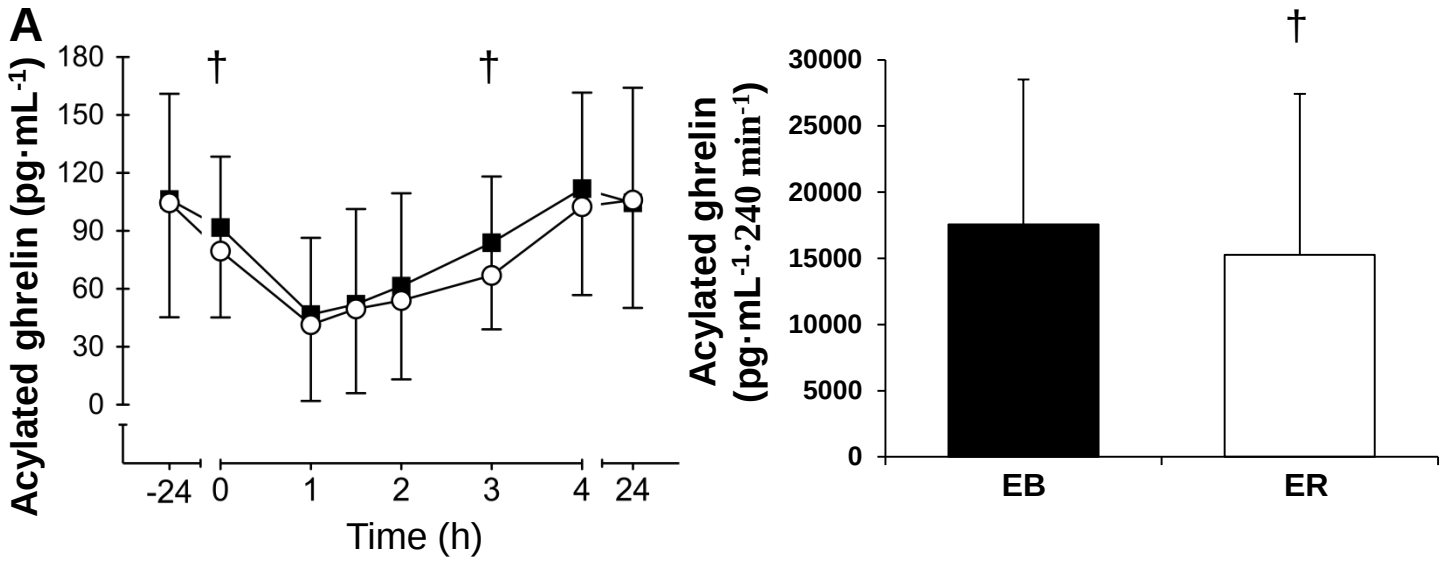
Blood sample

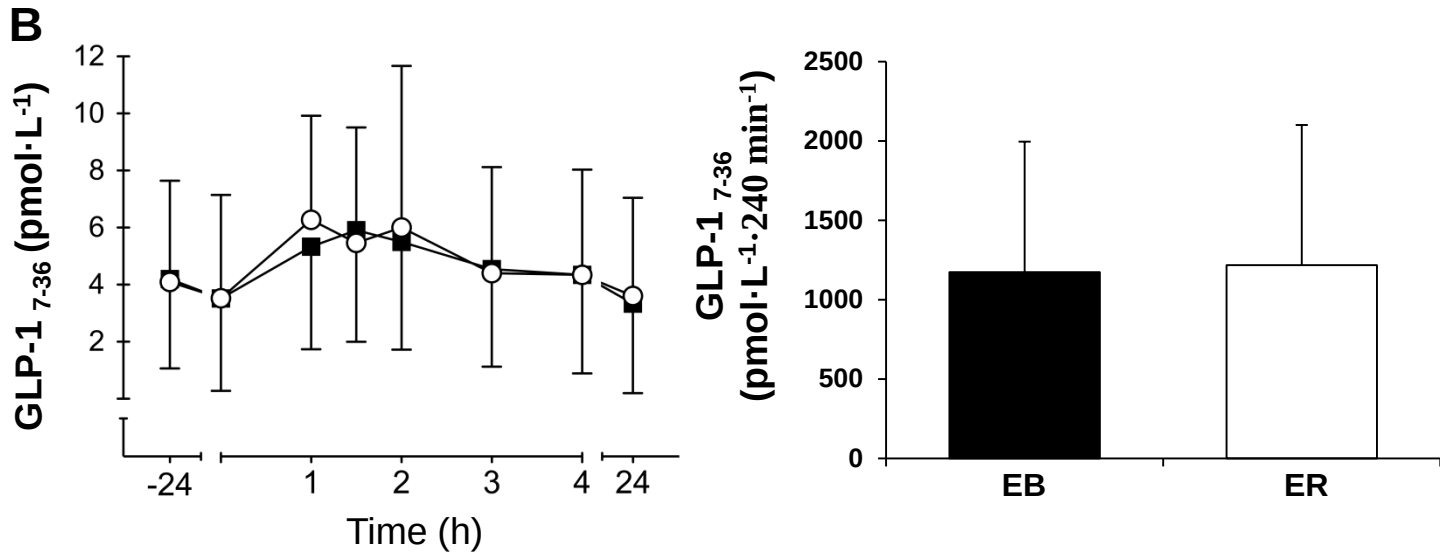
Expired air sample

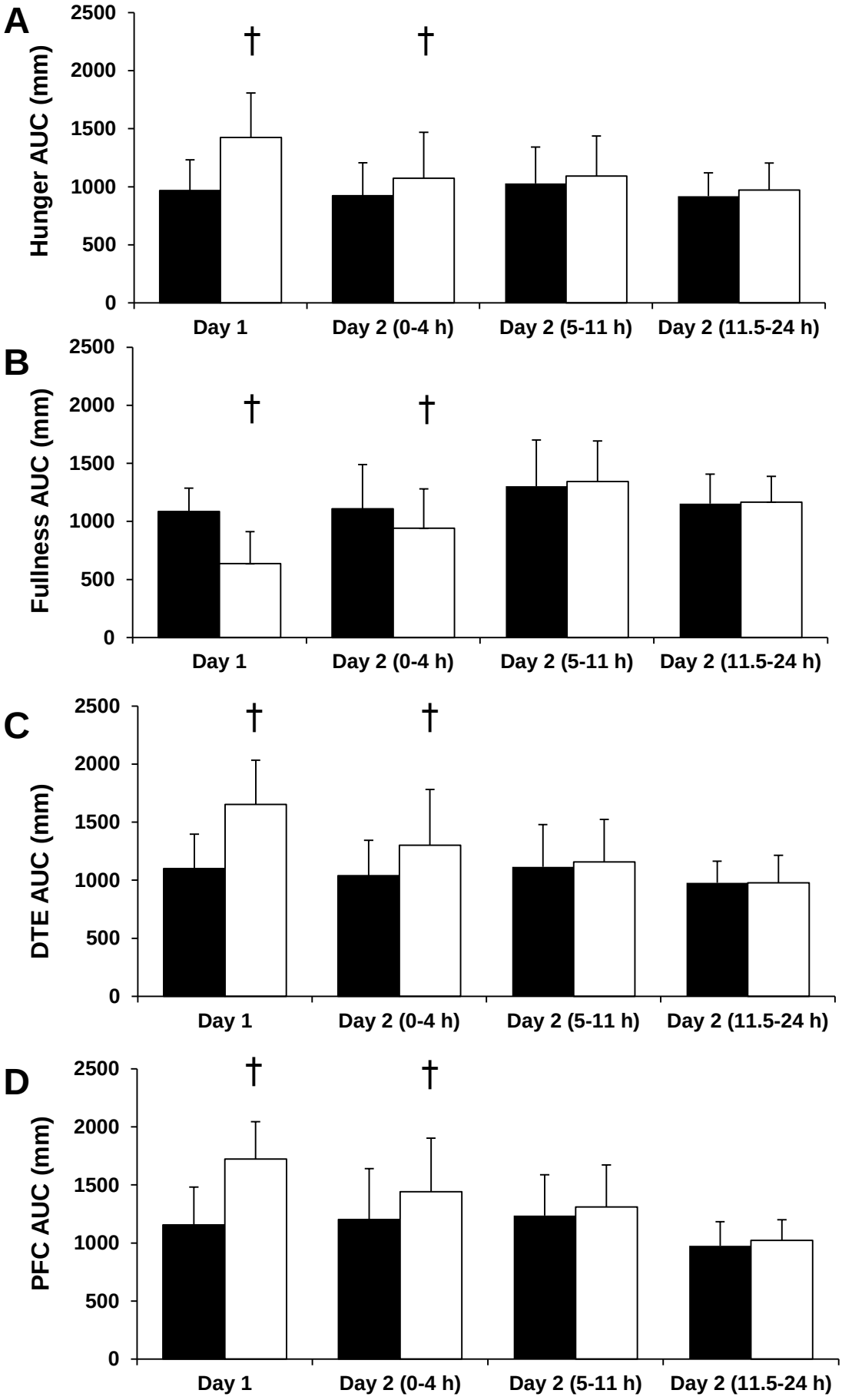
Subjective appetite questionnaire


A
Energy Expenditure (kJ·min-1)
8
7
6
5
4
3
-24
0
2
4
6
8
10
12
24
Time (h)
B
Carbohydrate oxidation (g·min-1)
Fat oxidation (g·min-1)
†††††
††
††
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.12
0.10
0.08
0.06
0.04
0.02
-24
2
4
6
8
10
12
24
Time (h)
C
Energy Expenditure (kJ·240 min-1)
3000
2500
2000
1500
1000
500
0
†
0-4 h
4.5-11 h
D
Carbohydrate oxidation (g·240 min-1)
120
100
80
60
40
20
0
†
†
0-4 h
4.5-11 h
Fat oxidation (g·240 min-1)
50
40
30
20
10
0
†
†
0-4 h
4.5-11 h


A
Glucose (mmol·L-1)
12
10
8
6
4
†
† †
-24
0
1
2
3
4
24
Time (h)
Glucose (mmol·L-1·240 min-1)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
†
EB
ER
B
Insulin (pmol·L-1)
700
600
500
400
300
200
100
0
-24
0
1
2
3
4
24
Time (h)
Insulin (pmol·L-1·240 min-1)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
(P=0.06)
EB
ER
C
NEFA (mmol·L-1)
1.0
0.8
0.6
0.4
0.2
0.0
† †
-24
0
1
2
3
4
24
Time (h)
NEFA (mmol·L-1·240 min-1)
80
70
60
50
40
30
20
10
0
†
EB
ER


A
Acylated ghrelin (pg·mL-1)
180
150
120
90
60
30
0
†
†
-24
0
1
2
3
4
24
Time (h)
Acylated ghrelin (pg·mL-1·240 min-1)
30000
25000
20000
15000
10000
5000
0
†
EB
ER


B
GLP-1 7-36 (pmol·L-1)
12
10
8
6
4
2
-24
1
2
3
4
24
Time (h)
GLP-1 7-36 (pmol·L-1·240 min-1)
2500
2000
1500
1000
500
0
EB
ER


A
Hunger AUC (mm)
2500
2000
1500
1000
500
0
†
†
Day 1
Day 2 (0-4 h)
Day 2 (5-11 h)
Day 2 (11.5-24 h)
B
Fullness AUC (mm)
2500
2000
1500
1000
500
0
†
†
Day 1
Day 2 (0-4 h)
Day 2 (5-11 h)
Day 2 (11.5-24 h)
C
DTE AUC (mm)
2500
2000
1500
1000
500
0
†
†
Day 1
Day 2 (0-4 h)
Day 2 (5-11 h)
Day 2 (11.5-24 h)
D
PFC AUC (mm)
2500
2000
1500
1000
500
0
†
†
Day 1
Day 2 (0-4 h)
Day 2 (5-11 h)
Day 2 (11.5-24 h)