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**No Effect of 24 h severe energy restriction on appetite regulation and *ad-libitum* energy intake in overweight and obese males**

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**Running title:** Severe energy restriction and appetite

**Key words:** appetite hormones; energy balance; calorie restriction; intermittent fasting; alternate day fasting

**Abstract**

**Background/ Objectives:** Long-term success of weight loss diets might depend on how the appetite regulatory system responds to energy restriction. This study determined the effect of 24 h severe energy restriction on subjective and hormonal appetite regulation, subsequent *ad-libitum* energy intake and metabolism.

**Subjects/ Methods:** In randomised order, eight overweight or obese males consumed a 24 h diet containing either 100% (12105 (1174 kJ; EB) or 25% (3039 (295) kJ; ER) of estimated daily energy requirements (EER). An individualised standard breakfast containing 25% of EER (3216 (341) kJ) was consumed the following morning and resting energy expenditure, substrate utilisation, and plasma concentrations of acylated ghrelin, GLP-1<sub>7-36</sub>, GIP<sub>1-42</sub>, glucose, insulin and NEFA were determined for 4 h after-breakfast. *Ad-libitum* energy intake was assessed in the laboratory on day 2 and via food records on day 3. Subjective appetite was assessed throughout.

**Results:** Energy intake was not different between trials for day 2 (EB: 14946 (1272) kJ; ER: 15251 (2114) kJ;  $P=0.623$ ), day 3 (EB: 10580 (2457) kJ; 10812 (4357) kJ;  $P=0.832$ ) or day 2 and 3 combined ( $P=0.693$ ). Subjective appetite was increased during ER on day 1 ( $P<0.01$ ), but was not different between trials on day 2 ( $P>0.381$ ). Acylated ghrelin, GLP-1<sub>7-36</sub>, and insulin were not different between trials ( $P>0.104$ ). Post-breakfast AUC for NEFA ( $P<0.05$ ) and GIP<sub>1-42</sub> ( $P<0.01$ ) were greater during ER compared to EB. Fat oxidation was greater ( $P<0.01$ ) and carbohydrate oxidation was lower ( $P<0.01$ ) during ER, but energy expenditure was not different between trials ( $P=0.158$ ).

**Conclusions:** These results suggest that 24 h severe energy restriction does not affect appetite regulation or energy intake in the subsequent 48 h. This style of dieting may be

conducive to maintenance of a negative energy balance by limiting compensatory eating behaviour, and therefore may assist with weight loss.

## Introduction

Overweight and obesity are positively associated with several chronic diseases, and consequently represent a considerable health and economic burden<sup>1,2</sup>. In these populations a weight loss of >5% body mass reduces the prevalence of some of these chronic diseases<sup>3</sup>. Traditional weight loss diets involve continuous daily energy restriction to induce a moderate daily energy deficit. This style of dieting is successful in some dieters, and typically results in long term weight loss of >5% body mass in approximately 30-40% of dieters<sup>4-6</sup>. One problem with such diets is thought to be the requirement for daily adherence to the diet in order to create a sufficiently large energy deficit to induce weight loss<sup>7</sup>. More recently, intermittent severe energy restriction has been proposed as an alternative to daily energy restriction<sup>8</sup>. This style of dieting involves severely restricting energy intake intermittently (1-4 days a week), with *ad-libitum*<sup>9,10,11</sup> or adequate<sup>12,13</sup> energy intake on other days. These studies have demonstrated that under tightly controlled conditions, intermittent severe energy restriction can achieve 4-8% weight loss in 8-24 weeks<sup>9,10,11,12,13</sup>.

A few studies have examined the effects of an acute period of severe energy restriction on *ad-libitum* energy intake in lean populations<sup>14,15,16,17</sup>. Generally, a small increase in energy intake has been observed in the days after a period of severe or complete energy restriction<sup>14,15,16</sup>. However, the increased energy intake is insufficient to compensate for the energy omitted, and consequently an energy deficit is induced, although this has not been examined in overweight or obese populations.

Energy balance is thought to be regulated, in part, by alterations in gastrointestinal hormone (orexigenic and anorexigenic) profiles, which act to influence appetite and correct perturbations in energy balance<sup>18,19</sup>. Despite this, little is known about how appetite hormone profiles are affected by severe energy restriction. Fasting concentrations of the orexigenic hormone ghrelin do not change in response to 48-96 h severe energy restriction<sup>16,20,21</sup> or chronic intermittent severe energy restriction<sup>13,22</sup>. Ghrelin is suppressed after food intake and concentrations return to fasting levels between meals<sup>18</sup>. The response of ghrelin, as well as other appetite hormones to feeding might play an important role in post-meal satiety and/ or subsequent meal initiation<sup>23</sup>. How the appetite regulatory system responds to acute periods of severe energy restriction has not been previously reported, and this might help to determine whether this style of dieting would be successful outside of rigid experimental control. In addition, the effects of severe energy restriction on *ad-libitum* energy intake in overweight/ obese individuals are unknown.

Therefore, the purpose of this study was to examine the effect of 24 h severe energy restriction (~25% of estimated energy requirement) on appetite regulation (hormonal and subjective) and *ad-libitum* energy intake compared to an adequate energy control trial.

## Methods

### *Subjects*

After institutional ethical approval, eight overweight/ obese (BMI  $\geq 28$  kg·m<sup>-2</sup>; Body fat >20%), but otherwise healthy males (age: 26 (4) y; weight 104.6 (17.6) kg; height: 1.82 (0.06) m; BMI: 32 (4) kg·m<sup>-2</sup>; body fat: 28 (4) %) provided written consent and completed this study. Subjects were not restrained, disinhibited or hungry eaters<sup>(24)</sup>, had been weight

stable for >6 months and were not currently dieting. Sample size was estimated from energy intake data from a similar study<sup>14</sup> and from unpublished energy intake data from our laboratory using the same *ad-libitum* meals, which provided a between group correlation of 0.83 (G\*Power 3.1.6; Dusseldorf, Germany). Using an  $\alpha$  of 0.05 and  $\beta$  of 0.2, it was determined 7 subjects would be required to reject the null hypothesis. Therefore we recruited 8 subjects to counterbalance the study and ensure an adequate sample size for the primary outcome (i.e. energy intake).

### *Study design*

Subjects completed a 1-day preliminary trial, during which height, weight and body fat percentage<sup>25</sup> were measured, before they were familiarised with the *ad-libitum* meals and blood sampling procedures. Subjects then completed two 3-day experimental trials in randomised, crossover, counterbalanced order, separated by  $\geq 14$  days. Each trial consisted of a 24 h dietary intervention period where subjects received 100% (i.e. energy balance; EB) or 25% (i.e. energy restriction; ER) of their estimated energy requirements (EER), followed by two days where dietary intake, behavioural and metabolic responses were measured (Figure 1).

### *Pre-trial standardisation*

Dietary intake and physical activity were recorded during the 48 h before the first experimental trial and these patterns were replicated before the second trial. Alcohol consumption and strenuous exercise were not permitted during this 48 h pre-trial period or during trials.

### *Protocol*

For each trial, subjects attended the laboratory on two consecutive mornings, arriving via motorised transport at ~07:30 after a  $\geq 10$  h fast. On day 1, blood (by venepuncture of an antecubital/ forearm vein) and expired air samples were collected and subjective appetite assessed (-24 h). Subjects were provided food and drink for the day, along with instructions for when to consume each item, and left the laboratory at ~08:30. Upon arrival on day 2, a cannula was inserted into an antecubital/ forearm vein and measurements made on day 1 were repeated (0 h). A standardised breakfast, providing 25% EER and consisting of white bread, jam, butter, cereal and semi-skimmed milk (3216 (341) kJ; 123 (12) g carbohydrate; 21 (2) g protein; 20 (3) g fat; 4 (1) g fibre) was consumed over 20 min. Subjects then rested in the laboratory, with blood and expired air samples collected and subjective appetite assessed periodically after breakfast. After the 4 h sample, the cannula was removed and an *ad-libitum* multi-item lunch was provided (4-4.5 h). After lunch, subjects left the laboratory, but were not permitted to consume any food or drink, with the exception of *ad-libitum* water and a standardised yoghurt and cereal bar snack (1135 (235) kJ; 33 (7) g carbohydrate; 5 (1) g protein; 13 (3) g fat; 1 (0) g fibre) at ~16:00 (8 h). Subjects returned at ~19:00 and were provided with an *ad-libitum* single-item dinner (11-11.5 h), after which they left the laboratory and were instructed not to consume any food or drink (other than water in the evening) until 08:00 the following morning (24 h). At 08:00 on day 3, subjective appetite was assessed (24 h) and subjects then completed a weighed food record for the rest of the day (24-48 h).

#### *Standardised diet preparation*

Diets contained palatable, recognisable foods, and were tailored to individual preferences. EER was determined by multiplying predicted resting metabolic rate<sup>26</sup> by a sedentary physical activity level of 1.4. The EB diet provided 100% of EER, distributed into four meals (Table 1): breakfast (20%; 08:00); lunch (30%; 12:00); snack (10%; 16:00); and dinner (40%;

19:00). The ER diet provided 25% of EER, distributed in to two meals (Table 1): lunch (34%; 12:00); and dinner (66%; 19:00); and a water only breakfast (0%; 08:00) provided isovolume to the EB breakfast water content. Additional water intake was prescribed at  $35 \text{ mL}\cdot\text{kg}^{-1}$  body mass (3661 (606) mL) and was evenly distributed throughout the day. Subjects consumed similar foods on day 1 during EB and ER. Due to the beneficial effects of dietary protein on satiety and preservation of fat-free mass during energy restriction<sup>27</sup>, the ER diet on day 1 was created by removing/ reducing high carbohydrate and high fat foods from the EB diet (i.e. pasta, bread, mayonnaise and snack foods).

### *Energy intake*

*Ad-libitum* meals were provided in excess of expected consumption and subjects were told that more food was available on request. The multi-item lunch meal consisted of cooked meats, bread, butter, mayonnaise, salad, fruit, crisps and biscuits. The dinner meal consisted of pasta, tomato sauce and olive oil, was homogenous in nature providing  $6.19 (0.02) \text{ kJ}\cdot\text{g}^{-1}$  (12, 68, 18 and 2 % of energy provided by protein, carbohydrate, fat and fibre, respectively), and was served as previously described<sup>28</sup>. Meals were served in an isolated feeding laboratory, with no social-interaction permitted. Subjects were given 30 min to consume each meal and were explicitly instructed to eat until they felt 'comfortably full and satisfied'. Food consumed was quantified by weighing before and after the meal, with the energy and macronutrient content of foods ascertained from manufacturer values. Food records completed on day 3 were analysed from manufacturer values (where possible) or using NetWisp 4.0 (Netwisp Inc, Chicago, USA).

### *Energy expenditure and substrate oxidation*

Ten min expired air samples were collected after 20 min of supine rest as described by Compher<sup>29</sup>. The first 5 min of each sample was discarded, with the second 5 min collected



into a Douglas bag, and analysed for O<sub>2</sub> and CO<sub>2</sub> 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 utilisation were then calculated<sup>30</sup>.

### *Subjective appetite*

Hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) were assessed on 100 mm visual analogue scales 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; at 0, 0.5, 1, 2, 3, 4, 4.5, 5, 6, 8, 8.25, 11, 11.5 h on day 2; and 24 h (pre-breakfast) on day 3. Verbal anchors 'not at all/ no desire at all/none at all' and 'extremely/a lot' were placed at 0 and 100 mm, respectively.

### *Blood sampling and analysis*

Blood samples (15 mL) were drawn after 30 min of supine rest, dispensed into tubes containing EDTA (1.75 mg·mL<sup>-1</sup>), and treated for the determination of active glucagon-like peptide-1 (GLP-1<sub>7-36</sub>) and acylated ghrelin concentrations, as previously described<sup>31</sup>. Treatment for determination of active glucose-dependant insulinotropic peptide (GIP<sub>1-42</sub>) concentration was identical to GLP-1<sub>7-36</sub>. Plasma was separated by centrifugation (15 min, 1750 g, 4°C). Plasma GLP-1<sub>7-36</sub> (Merck Millipore, Watford, UK), GIP<sub>1-42</sub> (Immuno-Biological Laboratories Ltd, Minneapolis, USA), acylated ghrelin (Bioquote Ltd, York, UK), and insulin (Merckodia, Uppsala, Sweden) concentrations were determined by ELISA. Plasma glucose (Horiba, Northampton, UK) and non-esterified fatty acid (NEFA; Randox Laboratories Ltd, Crumlin, UK) concentrations were determined by colorimetric assays (. Two mL of whole blood was used for determination of haemoglobin (via the

cyanmethaemoglobin method) and haematocrit (via microcentrifugation), and used to estimate changes in plasma volume relative to baseline<sup>32</sup>.

### *Statistical analysis*

Data were analysed using SPSS 21.0 (Somers, NY, USA). Correction of hormone concentrations for plasma volume changes did not alter results, so the unadjusted values are presented. All data were checked for normality using a Shapiro-Wilk test. Total area under the curve (AUC) and incremental area under the curve (iAUC) values were calculated using the trapezoidal method and were analysed using a t-test or Wilcoxon signed-rank test, as appropriate. AUC was calculated for the response to the standardised breakfast (0-4 h) for all variables, as well as for day 1 (-24-0 h) and the period post-lunch on day 2 (4.5-11.5 h) for subjective appetite sensations. Data containing two factors were analysed using two-way repeated measures ANOVA, followed by paired t-tests or Wilcoxon signed-rank tests, as appropriate. The Holm-Bonferroni adjustment was used to control the familywise error rate. Data sets were determined to be significantly different when  $P < 0.05$ . Data are presented as mean (SD) unless otherwise stated.

## **Results**

### *Energy intake*

There was no difference between trials for *ad-libitum* energy intake at lunch (EB: 5445 (792) kJ; ER: 5731 (1663) kJ;  $P=0.558$ ) and dinner (EB: 5149 (1070) kJ; ER: 5169 (1141) kJ;  $P=0.912$ ) on day 2. Furthermore, total *ad-libitum* energy intake on day 2 ( $P=0.623$ ), day 3 ( $P=0.832$ ) or day 2 and 3 combined ( $P=0.693$ ) was not different between trials (Table 1). Consequently, the energy deficit created on day 1 was maintained and total energy intake

over the 3 day trial was 11567 (2710) kJ greater during EB ( $P>0.0001$ ). There was also no difference in *ad-libitum* protein, carbohydrate, fat or fibre intake during day 2 ( $P>0.192$ ), day 3 ( $P>0.255$ ) or day 2 and 3 combined ( $P>0.326$ ).

#### *Energy expenditure and substrate oxidation*

There was an effect of time ( $P<0.0001$ ), but no trial ( $P=0.094$ ) or interaction ( $P=0.571$ ) effects for energy expenditure (Figure 2A). For carbohydrate and fat oxidation, there were time ( $P<0.001$ ), trial ( $P<0.05$ ) and interaction effects ( $P<0.05$ ) (Figure 2B). Carbohydrate oxidation was lower ( $P<0.01$ ) and fat oxidation higher ( $P<0.001$ ) at 1 h during ER compared to EB. Post-breakfast AUC ( $P=0.158$ ; Figure 2C) and iAUC (EB: 197 (98) kJ·240 min<sup>-1</sup>; ER: 242 (82) kJ·240 min<sup>-1</sup>;  $P=0.436$ ) was not different between trials for energy expenditure. AUC was lower for carbohydrate oxidation ( $P<0.01$ ) and higher for fat oxidation ( $P<0.01$ ; Figure 2D) during ER, but iAUC was not different for carbohydrate (EB: 19.1 (9.6) g·240 min<sup>-1</sup>; ER: 18.6 (13.3) g·240 min<sup>-1</sup>;  $P=0.939$ ) or fat (EB: 3.9 (3.2) g·240 min<sup>-1</sup>; ER: 4.0 (4.9) g·240 min<sup>-1</sup>;  $P=0.965$ ) oxidation.

#### *Blood parameters*

For plasma glucose concentration (Figure 3A), there were time ( $P<0.0001$ ) and interaction ( $P<0.05$ ) effects, but no trial effect ( $P=0.837$ ). Plasma glucose concentration was greater at 4 h during EB than ER ( $P<0.05$ ). There was a main effect of time ( $P<0.0001$ ), but no trial ( $P=0.499$ ) or interaction ( $P=0.787$ ) effects for plasma insulin concentration (Figure 3B). Post-breakfast AUC for plasma glucose ( $P=0.938$ ) and insulin ( $P=0.359$ ) concentrations were not different between trials. iAUC for plasma glucose (EB: 171 (157) mmol·L<sup>-1</sup>·240 min<sup>-1</sup>; ER: 218 (156) mmol·L<sup>-1</sup>·240 min<sup>-1</sup>;  $P=0.357$ ) and insulin (EB: 12112 (9097) pmol·L<sup>-1</sup>·240 min<sup>-1</sup>; ER: 11213 (7973) pmol·L<sup>-1</sup>·240 min<sup>-1</sup>;  $P=0.518$ ) were not different between trials. Plasma insulin and glucose concentrations peaked 1 h after breakfast in both trials, decreasing

thereafter. There were time ( $P<0.0001$ ), trial ( $P<0.05$ ) and interaction ( $P<0.0001$ ) effects for plasma NEFA concentration (Figure 3C). Plasma NEFA concentration was greater at 0 and 0.5 h during ER ( $P<0.05$ ). Post-breakfast AUC ( $P<0.05$ ) and iAUC (EB:  $61 (24) \text{ mmol}\cdot\text{L}^{-1}\cdot 240 \text{ min}^{-1}$ ; ER:  $96 (18) \text{ mmol}\cdot\text{L}^{-1}\cdot 240 \text{ min}^{-1}$ ;  $P<0.05$ ) was greater during ER compared to EB. Plasma NEFA concentration peaked at 0 h in both trials, decreasing thereafter.

For plasma acylated ghrelin concentration (Figure 4A), box plot analysis revealed one consistently outlying subject, exhibiting concentrations  $\sim 13$  SD greater than the mean of the 7 other subjects. Therefore, this subject was removed from the analysis. For acylated ghrelin concentration, there was a time effect ( $P<0.001$ ), but no trial ( $P=0.265$ ) or interaction ( $P=0.619$ ) effects. Post-breakfast AUC ( $P=0.109$ ) and iAUC (EB:  $4066 (4979) \text{ pg}\cdot\text{mL}^{-1}\cdot 240 \text{ min}^{-1}$ ; ER:  $4587 (5829) \text{ pg}\cdot\text{mL}^{-1}\cdot 240 \text{ min}^{-1}$ ;  $P=0.431$ ) was not different between trials. Plasma acylated ghrelin concentration was suppressed after breakfast in both trials, returning to fasting levels by 4 h. For plasma GLP-1<sub>7-36</sub> concentration (Figure 4B), there was a time effect ( $P<0.0001$ ) but no trial ( $P=0.162$ ) or interaction ( $P=0.119$ ) effects. Post-breakfast AUC ( $P=0.217$ ) and iAUC (EB:  $456 (268) \text{ pmol}\cdot\text{L}^{-1}\cdot 240 \text{ min}^{-1}$ ; ER:  $637 (157) \text{ pmol}\cdot\text{L}^{-1}\cdot 240 \text{ min}^{-1}$ ;  $P=0.105$ ) were not different between trials. Plasma GLP-1<sub>7-36</sub> peaked at 1.5 h in EB and 0.5 h in ER, decreasing thereafter. For plasma GIP<sub>1-42</sub> concentration (Figure 4C), there were time ( $P<0.0001$ ) and trial ( $P<0.05$ ) effects, but no interaction effect ( $P=0.157$ ). Post-breakfast AUC ( $P<0.01$ ) and iAUC (EB:  $7435 (1069) \text{ pmol}\cdot\text{L}^{-1}\cdot 240 \text{ min}^{-1}$ ; ER:  $9502 (1670) \text{ pmol}\cdot\text{L}^{-1}\cdot 240 \text{ min}^{-1}$ ;  $P<0.01$ ) were greater during ER compared to EB. Plasma GIP<sub>1-42</sub> peaked at 2 h during EB and 1 h during ER, decreasing thereafter.

#### *Subjective appetite sensations*

AUC for Hunger, DTE and PFC were greater, whilst AUC for fullness was lower during day 1 ( $P<0.01$ ), with no other differences in appetite sensations ( $P>0.381$ ; Figure 5).

### Body mass

Morning body mass on day 1 and 2, respectively was 104.4 (18.0) kg and 103.2 (17.9) kg during ER and 104.4 (18.3) kg and 104.2 (18.2) kg during EB. There were time ( $P<0.0001$ ) and interaction ( $P<0.0001$ ) effects for body mass with greater body mass loss from day 1 to day 2 during ER ( $P<0.0001$ ). Compared to day 1, body mass was reduced on day 2 during ER ( $P<0.0001$ ), but not EB ( $P=0.126$ ).

### Discussion

This study found that, following a single episode of severe energy restriction, overweight and obese individuals did not experience elevated appetite in the subsequent 24 h, and there was no change in resting or postprandial appetite hormone profiles. In addition, there was no increase in *ad-libitum* energy intake during the subsequent 48 h, suggesting that 24 h severe energy restriction may be an effective method of reducing energy intake in overweight and obese males, without any counter-regulatory effects on appetite.

In the current study, overweight and obese individuals did not adjust their energy intake in response to 24 h of severe energy restriction. Subjects consumed a similar amount of energy during days 2 and 3, irrespective of their energy intake on day 1. Consequently, the energy deficit created during day 1 on the ER trial was maintained. This is similar to previous studies in lean individuals, investigating 24-48 h periods of complete<sup>14,17</sup> or severe (provided 40% EER)<sup>15</sup> energy restriction. These studies reported either no compensation<sup>17</sup> or partial compensation<sup>14,15</sup> in the 1-4 days after the period of energy restriction. Taken together with findings from the current study, these studies demonstrate that energy intake is not accurately adjusted in the short term, in response to an acutely induced severe energy deficit. Therefore, this might represent a viable strategy for reducing energy intake.

In the current study, subjects reported greater hunger, DTE, PFC and lower fullness on day 1, during ER compared to EB. This is expected given the disparate energy intakes between trials on this day, and has previously been reported during 36 h complete energy restriction compared to an adequate energy diet<sup>14</sup>. In this study, consumption of an *ad-libitum* breakfast after energy restriction normalised subjective appetite<sup>14</sup>. In the current study, there was no difference in subjective appetite during day 2, suggesting that appetite is only transiently affected during a 24 h period of severe energy restriction, with no carry over onto subsequent days.

Acylated ghrelin is an orexigenic hormone that increases prior to a meal and might initiate food intake suggesting a role in energy balance homeostasis<sup>33</sup>. However, previous studies have reported that fasting ghrelin concentrations appear to be unchanged after 1-4 days energy restriction of varying severity<sup>16,20,21</sup>. In the current study, feeding reduced acylated ghrelin concentration, but fasting and postprandial acylated ghrelin concentrations were similar between trials, independent of whether subjects consumed 100 or 25 % of their estimated energy requirements during the previous 24 h. Doucet *et al.*<sup>21</sup> similarly observed no difference in ghrelin suppression in response to a standardised breakfast, before and after consumption of a moderately hypoenergetic diet (~70% EER) for 4 days. The anorexigenic hormone GLP-1<sub>7-36</sub> was also not different between trials. Intravenous infusion of GLP-1<sub>7-36</sub> has been shown to reduce appetite and food intake<sup>34</sup>, suggesting GLP-1<sub>7-36</sub> may be involved in satiation and satiety<sup>19</sup>. Fasting and postprandial GLP-1<sub>7-36</sub> concentrations are reduced after weight loss<sup>35,36</sup>, but fasting and postprandial GLP-1<sub>7-36</sub> concentrations were not different between trials in the current study. Taken together, both GLP-1<sub>7-36</sub> and acylated ghrelin may serve as feeding cues within day, but data from the current study suggest they are not altered after a single episode of severe energy restriction.

Given the proposed role of these hormones in appetite regulation, these findings may have important implications for energy balance homeostasis during chronic intermittent severe energy restriction. Considering there was also no difference in subjective appetite response between ER and EB, the current study suggests that 24 h severe energy restriction does not affect subjective or hormonal appetite regulation. These findings likely explain the lack of hyperphagia observed in the current study, and may at least partly explain the weight loss achieved and improved adherence to chronic intermittent severe energy restriction diets in overweight/ obese populations<sup>9,10,11,12,13</sup>.

In the current study, resting energy expenditure was unaffected by severe energy restriction, which is in line with findings from studies investigating short periods of complete energy restriction<sup>37,38,39</sup>. However, fasting and postprandial substrate metabolism was affected by 24 h of severe energy restriction, with fat oxidation greater and carbohydrate oxidation lower on day 2, during the ER trial. This is indicative of altered nutrient supply and/ or endogenous stores and has been reported previously<sup>37,38,39</sup>. Complete energy restriction for 24 h has been shown to greatly reduce liver glycogen<sup>40</sup>, but in the absence of exercise, muscle glycogen stores are largely preserved<sup>41</sup>. Although some carbohydrate was provided in the present study, it seems likely that this was not sufficient to meet the obligate requirement of this group of subjects<sup>42</sup>. Consequently this reduction in carbohydrate intake/ availability would stimulate lipolysis to provide substrate to preserve endogenous glycogen<sup>42</sup>. This is reflected in the greater plasma NEFA concentration during ER, which would increase fat oxidation and concomitantly reduce carbohydrate oxidation<sup>38</sup>.

These changes in substrate availability may have led to a slight alteration in glycaemic control. Whilst, there was no difference in glucose AUC, there appeared to be an altered pattern of postprandial glycaemia in response to the breakfast meal, evidenced by the observed interaction effect. Plasma glucose concentration was lower at 4 h during ER and

whilst there was no other significant difference between trials, there appeared to be some disturbance in glycaemic control during the first 2 h post-breakfast. Indeed, before correction for multiple comparisons, serum glucose concentration was higher at 1 h during ER compared to EB ( $P=0.04$ ). Prolonged complete energy restriction (i.e. starvation) is known to impair glycaemic control<sup>43</sup>, an effect that is likely attributable to increased plasma NEFA concentrations, which have been shown to reduce the rate of glucose uptake into muscle<sup>44,45</sup>. In addition, GIP<sub>1-42</sub> AUC was greater after ER compared to EB. GIP<sub>1-42</sub> and GLP-1<sub>7-36</sub> are incretin hormones, synthesised rapidly from the stomach in response to nutrient intake, and stimulate the release of insulin prior to nutrient absorption<sup>46</sup>. In the current study, despite elevated GIP<sub>1-42</sub> during ER, the insulinotropic response to the standardised breakfast was not different between trials. The incretin effect is known to be impaired in obese and insulin resistant individuals<sup>47</sup>, which might explain why there was an increase in GIP<sub>1-42</sub>, but not insulin after-breakfast. Although not an aim of the current study, these results suggest that severe energy restriction may impact glycaemic control, and whilst this study might be underpowered to elucidate the precise effects/ mechanisms, these results suggest this topic warrants further investigation.

A potential issue with intermittent severe energy restriction is whether the degree of energy restriction required for this type of dieting to be successful is achievable under free-living conditions. Whilst appetite is increased during a period of severe energy restriction, the current study suggests these feelings are transient and constrained to the day of severe energy restriction. This and a previous study<sup>14</sup> suggest that severe energy restriction does not lead to any increase in appetite sensations in the days after a 24 h period of severe energy restriction. Daily energy restriction is the traditional method of dietary induced weight loss<sup>48</sup>, however compliance to such diets may be compromised by continuous hunger and the need for daily adherence to the diet<sup>7</sup>. In theory, intermittent severe energy restriction might represent a more



flexible dietary strategy compared to daily energy restriction, and may facilitate better long term compliance by assisting with appetite regulation, although this theory remains to be tested.

Previous studies have demonstrated weight loss of 4-12% after 8-24 weeks of intermittent severe energy restriction<sup>9,10,11,12,13</sup>. In one study, weight loss was greater after 12 weeks intermittent severe energy restriction compared to isoenergetic daily energy restriction<sup>13</sup>. The current study observed no difference in subjective appetite, and no difference in resting or postprandial concentrations of the appetite hormones acylated ghrelin and GLP-1<sub>7-36</sub> after 24 h energy balance or severe energy restriction. These results suggest short periods of severe energy restriction may produce an appetite profile conducive to weight loss, but whether this appetite profile is maintained after long term exposure to intermittent severe energy restriction has yet to be determined. Whilst no change in fasting ghrelin concentration was reported after 16 weeks of intermittent severe energy restriction<sup>13</sup>, the dynamic response to feeding of appetite hormones after long term intermittent severe energy restriction is unknown.

The current study had the following limitations. The sample size for the study (n=8) was calculated to be sufficient to detect a difference in *ad-libitum* energy intake, however this sample size may be too small to detect differences in some blood parameters. This study also investigated a homogenous cohort of overweight/ obese, young (20-40 y) adult males, and it is not known whether these findings extend to females, lean individuals, or older populations. The energy expenditure assessment in the current study did not account for physical activity, and therefore the effect of severe energy restriction on this component of energy balance remains to be determined. Finally, whether the acute effects observed in the current study extend to the chronic intermittent severe energy restriction paradigm is unknown, with long term intervention studies required to determine this.

In conclusion, the results of this study demonstrate that subjective appetite is only transiently affected during, and not after severe energy restriction, and that fasting and postprandial appetite hormone profiles are unaffected by an acute 24 h period of severe energy restriction. In addition, no difference in energy intake was observed up to 48 h after 24 h severe energy restriction, thereby preserving the deficit induced by energy restriction. This is the first study to assess this in overweight/ obese subjects and suggests that 24 h of severe energy restriction induces an appetite response conducive to weight loss in these individuals, and may help explain findings from longer-term intervention studies.

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### **Declaration of interests**

The authors declare no conflicts of interest.

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**Table and Figure legends**

**Table 1.** Energy and macronutrient intake during each day of the experimental trial.

**Figure 1.** Schematic representation of study protocol

**Figure 2.** Line graphs represent energy expenditure (A) and substrate oxidation (B) during EB (■) and ER (○). Data points are means with vertical error bars representing standard deviation. Bar charts represent post-breakfast AUC for energy expenditure (C) and substrate oxidation (D) during EB (■) and ER (□). † indicates values are significantly different to EB ( $P<0.05$ ).

**Figure 3.** Line graphs represent glucose (A), insulin (B) and NEFA (C) concentrations, during EB (■) and ER (○). Bar charts represent post-breakfast AUC during EB (■) and ER (□). Data points are means with vertical error bars representing standard deviation. † indicates values are significantly different to EB ( $P<0.05$ ).

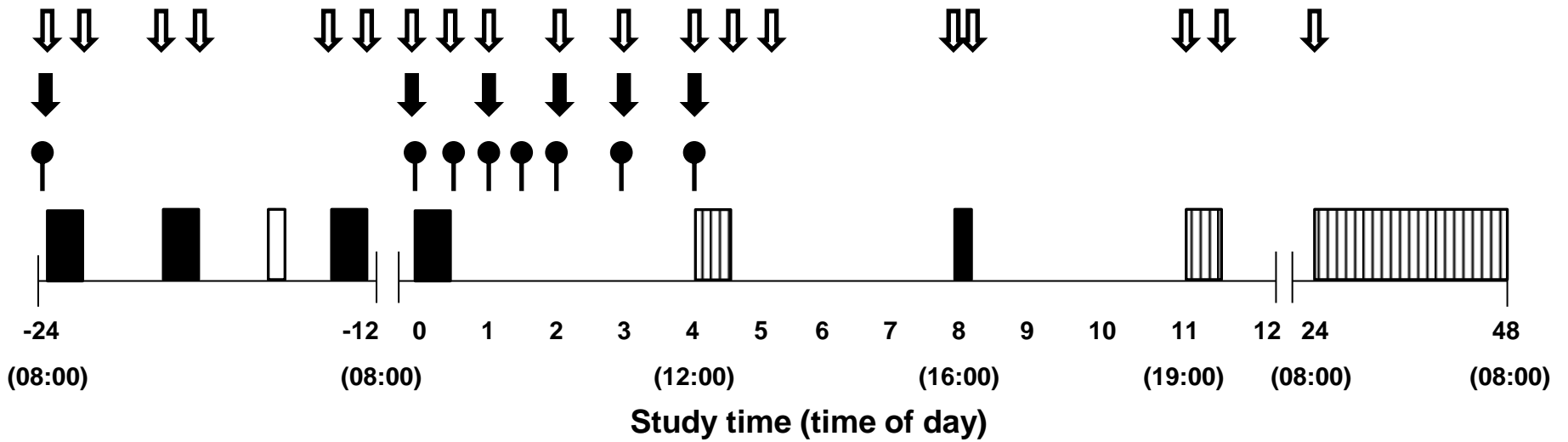
**Figure 4.** Line graphs represent acylated ghrelin (A), GLP-1<sub>7-36</sub> (B) and GIP<sub>1-42</sub> (C) concentrations, during EB (■) and ER (○). Bar charts represent post-breakfast AUC during EB (■) and ER (□). Data points are means with vertical error bars representing standard deviation. † indicates values are significantly different to EB ( $P<0.05$ ).







**Figure 5.** AUC for hunger (A), fullness (B), DTE (C), and PFC (D), on day 1, the morning of day 2 (0-4 h) and the afternoon of day 2 (4.5-11.5 h), during EB (■) and ER (□). Data points are means with vertical error bars representing standard deviation. † indicates values are significantly different to EB ( $P<0.05$ ).

**Table 1.** Energy and macronutrient intake during each day of the experimental trial.

	<b>Protein (g)</b>	<b>Carbohydrate (g)</b>	<b>Fat (g)</b>	<b>Fibre (g)</b>	<b>Energy (kJ)</b>
<i>Day 1</i>					
<b>Energy Balance</b>	125 (12)	381 (37)	91 (9)	14 (1)	12105 (1174)
<b>Energy Restriction</b>	78 (8) <sup>†</sup>	73 (7) <sup>†</sup>	12 (1) <sup>†</sup>	4 (0) <sup>†</sup>	3039 (295) <sup>†</sup>
<i>Day 2</i>					
<b>Energy Balance</b>	119 (21)	494 (52)	117 (14)	24 (3)	14946 (1272)
<b>Energy Restriction</b>	117 (24)	500 (52)	123 (29)	25 (4)	15251 (2114)
<i>Day 3</i>					
<b>Energy Balance</b>	105 (32)	310 (85)	91 (41)	19 (7)	10580 (2457)
<b>Energy Restriction</b>	133 (58)	318 (134)	83 (55)	20 (8)	10812 (4357)
<i>Daily averaged intake</i>					
<b>Energy Balance</b>	117 (12)	395 (39)	100 (14)	19 (3)	12543 (1174)
<b>Energy Restriction</b>	83 (25) <sup>†</sup>	273 (48) <sup>†</sup>	69 (27) <sup>†</sup>	15 (4) <sup>†</sup>	8688 (1922) <sup>†</sup>

<sup>†</sup> indicates significant difference to EB ( $P < 0.05$ ). Data are means (SD)



-  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

