Effect of 24-h severe energy restriction on appetite regulation and ad libitum energy intake in lean men and women[1,2]

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ABSTRACT

Background: Intermittent severe energy restriction (SER) can induce substantial weight loss, but the appetite regulatory responses to SER are unknown and may dictate long-term dietary adherence.
Objective: We determined the effect of 24-h SER on appetite regulation, metabolism, and energy intake.
Design: Eighteen lean men and women completed two 3-d trials in randomized, counterbalanced order. On day 1 subjects consumed standardized diets containing 100% (mean ± SD: 9.3 ± 1.3 MJ; energy balance) or 25% [2.3 ± 0.3 MJ; energy restriction (ER)] of energy requirements. On day 2, a standardized breakfast was consumed, with plasma concentrations of acylated ghrelin, glucagon-like peptide 1, insulin, glucose, and nonesterified fatty acids 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 by 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 the morning of day 2 ($P < 0.05$) but was not significantly different thereafter ($P > 0.145$). During ER, postprandial concentrations of acylated ghrelin were lower ($P < 0.05$), whereas glucose ($P < 0.05$) and nonesterified fatty acids ($P < 0.0001$) were higher. Postprandial glucagon-like peptide 1_{7-36} ($P = 0.784$) and insulin ($P = 0.06$) concentrations were not significantly different between trials. Energy expenditure was lower during ER in the morning ($P < 0.01$).
Conclusions: In lean young adults, 24-h SER 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 that intermittent SER might be useful to attenuate energy intake and control body weight in this population. This trial was registered at www.clinicaltrials.gov.uk as NCT02696772. *Am J Clin Nutr* doi: 10.3945/ajcn.116.136937.

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. Although the majority of weight-management research tends to focus on methods to assist obese individuals lose weight, there is research to suggest 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 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). Intermittent severe energy restriction (SER)[3] has been proposed as an alternative to daily energy restriction (6). This involves severely restricting energy intake intermittently (1–4 d/wk), 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 wk (7–11), which is comparable with weight loss reported from daily energy-restriction diets (6).

Studying the acute effects of SER may elucidate some of the mechanisms of action. Persistent hunger is often cited as a reason for poor adherence to weight-management regimens (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 short periods of SER. A recent study reported that 48 h of SER produced a postprandial appetite hormone profile that would be expected to suppress rather than stimulate appetite in male and female soldiers

(15), but the large exercise component and incorporation of meal replacement gels possibly limits the translation of these findings to weight-management settings.

The aim of this study was to examine the effect of 24 h of SER [providing 25% of estimated energy requirements (EERs)] on subjective and hormonal appetite regulation, as well as ad libitum food intake, compared with an adequate-energy control diet. We hypothesized that, relative to the control trial, acylated ghrelin response would be greater and GLP-1$_{7-36}$ reduced after 24 h of SER and that this would be concurrent with 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, United Kingdom. After institutional ethical approval, 10 healthy men and 8 healthy women (**Table 1**) provided written consent and completed the study. Subjects were not restrained, disinhibited, or hungry eaters (16), had been weight stable for >6 mo, and were not currently dieting. Female participants completed a menstrual cycle questionnaire and were tested during the postmenstruation follicular phase (~5–12 d after start of menstruation). Sample size was estimated from energy intake data from a similar study (17), data from our laboratory that used similar ad libitum meals (18), and an estimated between-group correlation of 0.5 (G*Power 3.1.6). Using an α of 0.05 and statistical power of 0.95, we determined that ≥16 subjects would be required to reject the null hypothesis.

Study design

During a 1-d 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-d experimental trials, administered in a crossover, randomized, counterbalanced order. Trials were separated by ≥14 d for men and exactly one menstrual cycle for women. On day 1 of each experimental trial, subjects received either 100% [energy balance diet (EB)] or 25% [energy restricted diet (ER)] of their EER. On days 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$_{7-36}$). The secondary outcome measures were glucose, insulin, nonesterified fatty acids (NEFAs), and expired gas measures.

TABLE 1
Baseline subject characteristics[1]

	Men ($n = 10$)	Women ($n = 8$)
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/m^2	24 ± 2	24 ± 2
Body fat, %	14 ± 4	27 ± 5

[1] Values are means ± SDs.

Pretrial standardization

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

Protocol

For each trial, subjects arrived at the laboratory via motorized transport at ~0730 on 3 consecutive mornings after a ≥10-h overnight fast, and after voiding nude body mass was measured (Adam Equipment Co.). On day 1, expired gas and blood (via venepuncture) samples were collected and subjective appetite assessed (~0800, −24 h). Subjects left the laboratory at ~0830 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 (~0800, 0 h). A standardized breakfast consisting of cereal, semiskimmed milk, white bread, butter, and jam (mean ± SD: 2.5 ± 0.3 MJ, 16 ± 2 g protein, 93 ± 13 g carbohydrate, 16 ± 2 g fat, and 3 ± 0 g fiber) and providing 25% EER was then consumed over 20 min. Subjects then rested in the laboratory with subjective appetite sensations and 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 (~1200–1230; 4–4.5 h). After lunch, subjects rested in the laboratory with further expired gas (at 5, 7, 9, and 11 h) and subjective appetite sensations collected (5, 6, 7, 8, 8.25, 9, 10, and 11 h). A standardized yogurt and cereal bar snack (0.9 ± 0.1 MJ, 4 ± 1 g protein, 25 ± 3 g carbohydrate, 10 ± 1 g fat, and 1 ± 0 g fiber) was consumed at ~1600 (8 h), and an ad libitum dinner was provided at ~1900–1930 (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 was assessed (~0800, 24 h), and an ad libitum porridge breakfast was provided at 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% at 0800), lunch (30% at 1200), afternoon snack (10% at 1600), and dinner (40% at 1900). During ER, 25% of EER was provided (Table 2), divided between lunch (34% at 1200) and dinner (66% at 1900), with a water-only breakfast (0% at 0800) provided isovolume to the water content of the breakfast provided in EB. Additional water intake was prescribed at 35 mL/kg 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
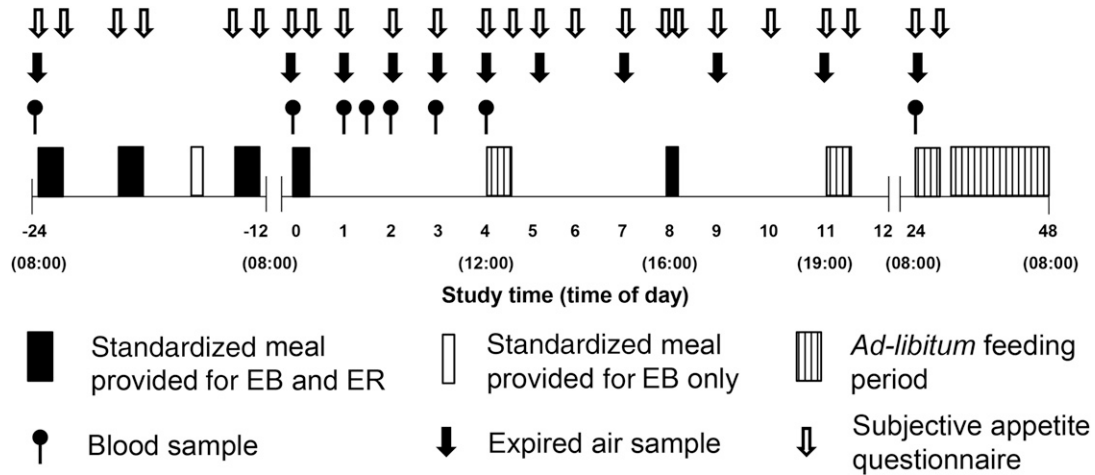
FIGURE 1 Schematic representation of the study protocol. EB, energy balance trial; ER, energy restriction trial.

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/g; 12%, 68%, 18%, and 2% of energy provided by protein, carbohydrate, fat, and fiber, respectively); and the homogenous breakfast consisted of porridge oats and semiskimmed milk (4.40 ± 0.05 kJ/g; 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 when possible or by using NetWisp dietary analysis software (Netwisp Inc.).

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 oxygen and carbon dioxide concentration (1400 series; Servomex), volume (Harvard Dry Gas Meter; Harvard Ltd), and temperature (Edale thermistor). 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 prebreakfast (−24 h), postbreakfast (−23.5 h), prelunch (−20 h), postlunch (−19.5 h), predinner (−13 h), and postdinner (−12.5 h) on day 1; prebreakfast (0 h), postbreakfast (0.33 h), and at 1, 2, 3, 4, 4.5, 5, 6, 7, 8, 8.25, 9, 10, 11, and 11.5 h on day 2; and prebreakfast (24 h) and postbreakfast (24.5 h) on day 3. Ratings were provided on 100-mm visual analog scales with verbal anchors "not at all/none at all/no desire at all" and "extremely/a lot" placed at 0 and 100 mm, respectively.

Blood sampling and analysis

Blood samples (15 mL) were drawn from an antecubital vein after 30 min of supine rest. Blood was dispensed into tubes containing EDTA (1.75 mg/mL) pretreated for the determination

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[†]
Fiber, 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] Values are means ± SDs. $n = 18$. [†]ER was significantly different from EB as determined by paired t test, $P < 0.05$. EB, energy balance trial; ER, energy restricted trial.

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of acylated ghrelin and GLP-1$_{7-36}$ concentrations as previously described (24), and plasma was separated by centrifugation (15 min; 1750 × g; 4°C). Concentrations of GLP-1$_{7-36}$, (CV: 4.8%; Merck Millipore), acylated ghrelin (CV: 3.7%; Bioquote Ltd.), and insulin (CV: 3.2%; Immunodiagnostics Systems) were determined by ELISA. The limit of detection for each variable was determined by the lowest standard provided in the ELISA kit, and this value was assigned to any measured concentration below this value per manufacturer instructions. Glucose (CV: 0.5%; Horiba) and NEFA (CV: 2.9%; Randox Laboratories Ltd.) concentrations were determined by colorimetric assay with the use of a benchtop analyzer (Pentra 400; Horiba). Two milliliters of whole blood was used for determination of hemoglobin (by using the cyanmethemoglobin method) and hematocrit (by using microcentrifugation) and used to estimate changes in plasma volume relative to baseline (25).

Statistical analysis

Data were analyzed by using SPSS 21.0 (Somers). Because of problems with blood sampling, blood samples were collected for only 16 of the 18 subjects (8 men and 8 women). For all other measures, $n = 18$. Using the change in plasma volume to correct blood variables did not alter the results, so the unadjusted values are presented. All data were checked for normality by using a Shapiro-Wilk test. Data containing two factors were analyzed by using a 2-way repeated-measures ANOVA followed by post hoc paired t tests or Wilcoxon's Signed Rank tests, as appropriate. The Holm-Bonferroni adjustment was used to control the family-wise error rate. Total AUC values were calculated by using the trapezoidal method and were analyzed by using a t test or Wilcoxon's 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, sex was entered as a between-subjects factor in repeated-measures ANOVA to test for sex-by-trial-by-time interactions and sex-by-trial interactions (AUC and energy intake). Data sets
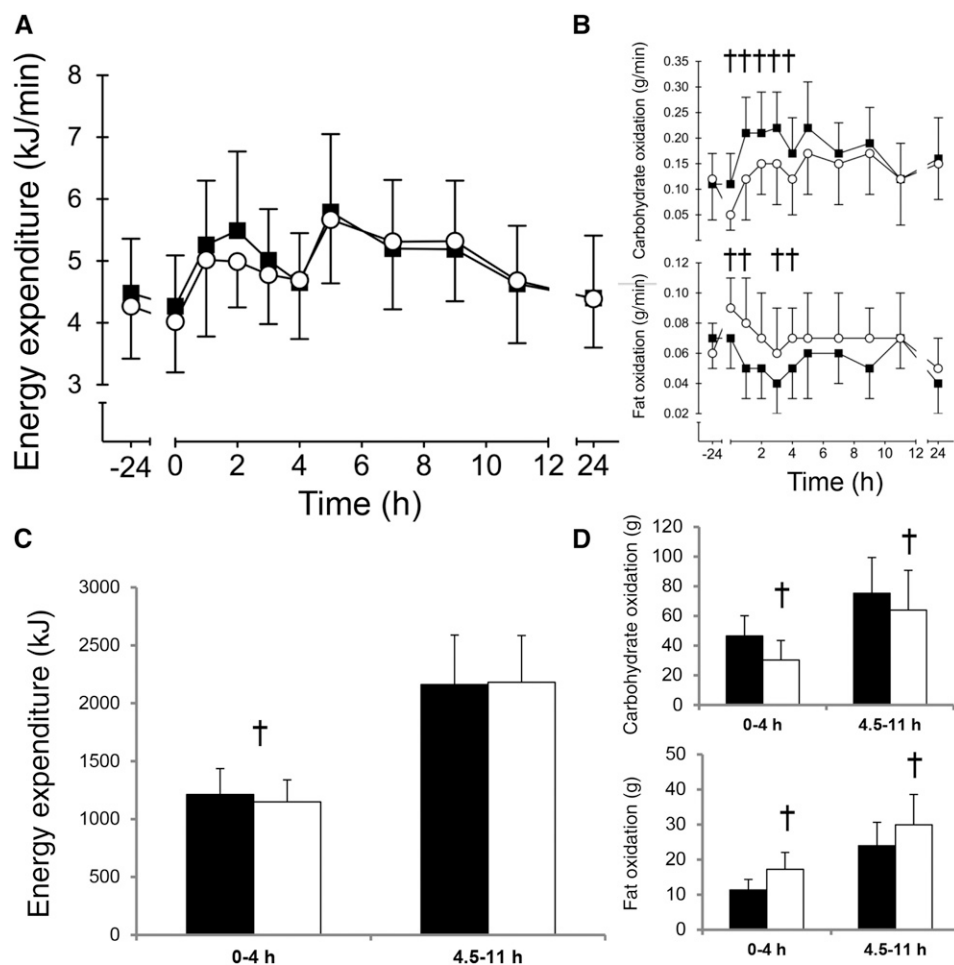
FIGURE 2 Energy expenditure (A) and substrate oxidation (B) on day 2 of the experimental trial, during the EB trial (■) and the ER trial (○). Data points are means with vertical error bars representing SDs ($n = 18$). Bar charts represent mean energy expenditure (C) and substrate oxidation (D) AUC during EB (■) and ER (□) with vertical error bars representing SD. 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 2-way repeated-measures ANOVA. †ER values were significantly different from EB values, determined by Bonferroni-Holm adjusted paired t test, $P < 0.05$. EB, energy balance; ER, energy restriction.

were determined to be significantly different when $P < 0.05$. Data are presented as means $\pm$ SDs unless otherwise stated.

RESULTS

Sex analysis

There were main effects of sex for some variables, with plasma NEFA concentration greater in woman ($P < 0.05$) 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 men. There were no sex-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 sex-by-trial interaction effect for fullness AUC between lunch and dinner on day 2 ($P < 0.05$) with fullness lower in men on ER than on EB ($P < 0.05$). There were no other sex-by-trial ($P < 0.274$) or sex-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 $\pm$ 1.5 MJ; ER: 4.8 $\pm$ 1.3 MJ; $P < 0.05$) and tended to be greater at dinner (EB: 4.3 $\pm$ 0.1 MJ; ER: 4.6 $\pm$ 1.2 MJ; $P = 0.056$) during ER. Therefore, total ad libitum energy intake on day 2 was 7% greater during ER than during EB ($P < 0.05$). On day 3, ad libitum energy intake was not significantly different at breakfast (EB: 2.2 $\pm$ 0.6 MJ; ER: 2.4 $\pm$ 0.5 MJ; $P = 0.162$), and there was no difference in reported energy intake over the remainder of the day (EB: 9.0 $\pm$ 3.0 MJ; ER: 8.5 $\pm$ 2.8 MJ; $P = 0.362$). Over the 2-d period, the increase in energy intake (0.5 $\pm$ 2.9 MJ) was only sufficient to replace $\sim$7% of the energy deficit created on day 1. Therefore, energy intake over the 3-d trial was 6.5 $\pm$ 3.3 MJ greater during EB ($P < 0.00001$; Table 2).
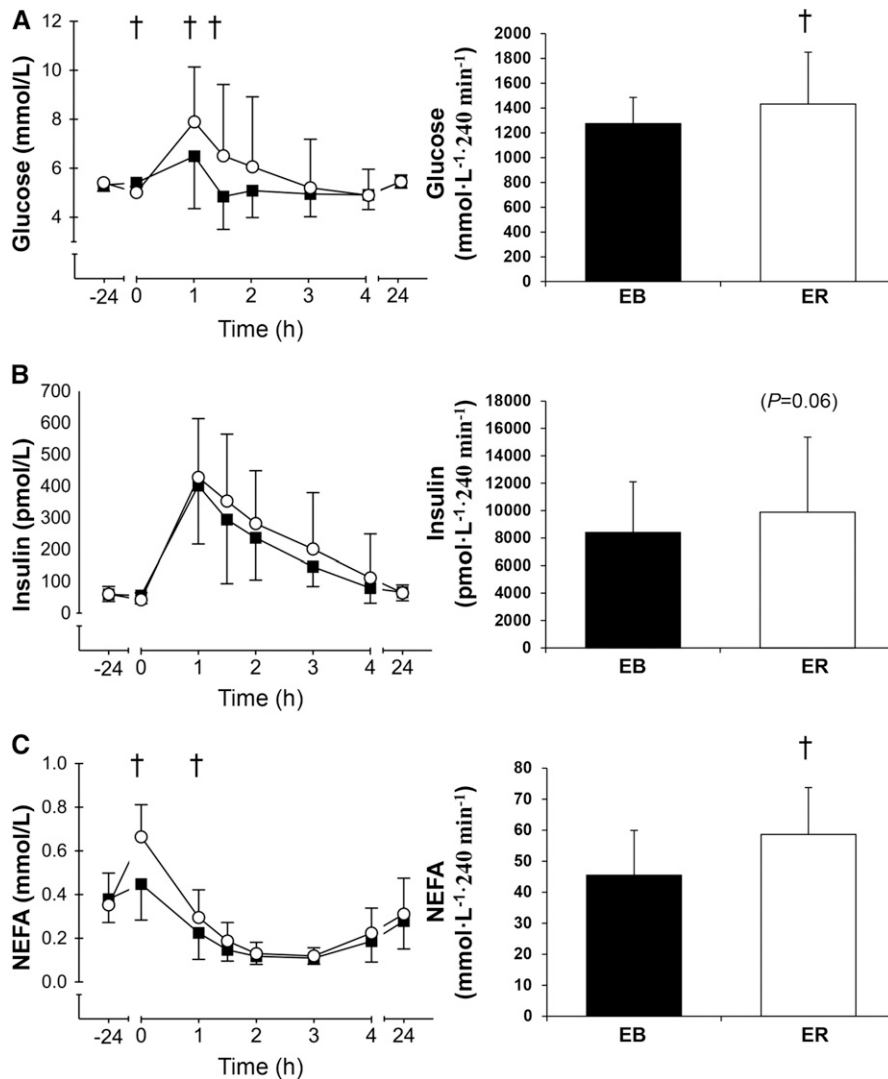
FIGURE 3 Plasma glucose (A), insulin (B), and NEFAs (C) during the EB trial (■) and ER trial (○). Data points are means with vertical error bars representing SDs ($n = 16$). Bar charts represent mean AUC response (0–4 h) to a 2.5 $\pm$ 0.3–MJ standardized breakfast during EB (■) and ER (□), with vertical error bars representing SDs. There were main effects of time for plasma glucose, insulin, and NEFAs (all $P < 0.0001$); a main effect of trial for plasma glucose and NEFAs (both $P < 0.05$) but not insulin ($P = 0.057$); and interaction effects for plasma glucose and NEFAs (both $P < 0.00001$) but not insulin ($P = 0.120$) examined by 2-way repeated-measures ANOVA. †ER values were significantly different from EB values, determined by Bonferroni-Holm adjusted paired t test, $P < 0.05$. EB, energy balance; ER, energy restriction; NEFA, nonesterified fatty acid.

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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**). Postbreakfast 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 than during EB. Postbreakfast AUC was lower for carbohydrate oxidation ($P < 0.00001$) and greater for fat oxidation ($P < 0.0001$; Figure 2) during ER. Furthermore, postlunch 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 and 1.5 h ($P < 0.05$) during ER. Plasma glucose AUC was greater during ER than during 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 and 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 than during 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$_{7-36}$, and plasma GLP-1$_{7-36}$ 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 postbreakfast on day 2 ($P < 0.05$). There were no differences in postlunch ($P > 0.145$) or overnight ($P > 0.214$) AUC for appetite sensations (**Figure 5**).

Body mass

Morning body mass on days 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 than during EB ($P < 0.001$), and body mass on day 2 was lower during ER than during EB ($P < 0.001$). Day 3 body mass was not significantly different between trials ($P = 0.594$).
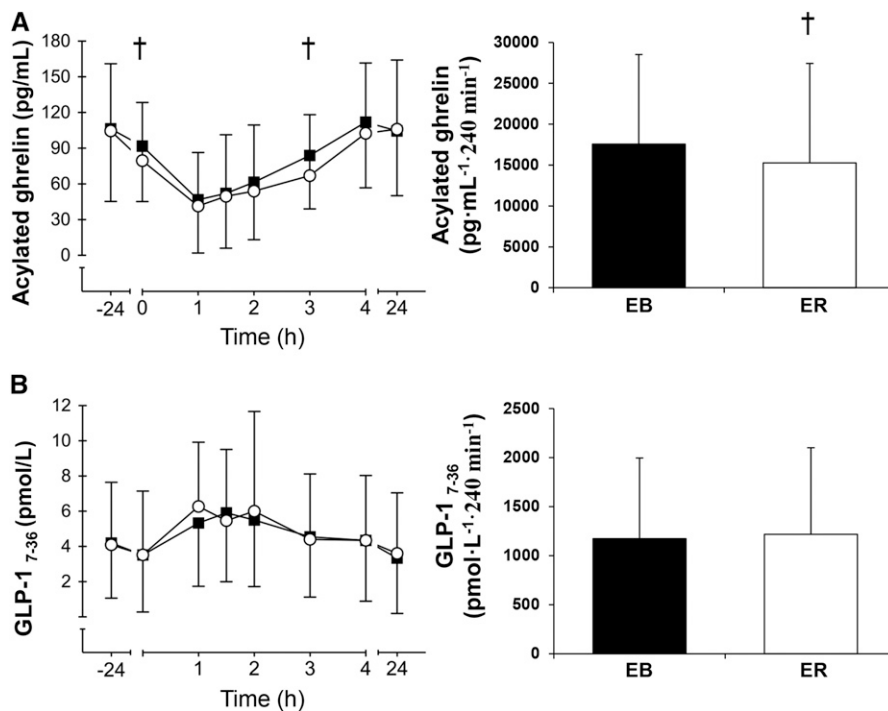
FIGURE 4 Plasma acylated ghrelin (A) and GLP-1$_{7-36}$ (B) during the EB trial (■) and ER trial (○). Data points are means with vertical error bars representing SDs ($n = 16$). Bar charts represent the mean AUC responses (0–4 h) to a 2.5 ± 0.3–MJ standardized breakfast during EB (■) and ER (□), with vertical error bars representing SDs. 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 2-way repeated-measures ANOVA. †ER values were significantly different from EB values, determined by Bonferroni-Holm adjusted paired t test, $P < 0.05$. EB, energy balance; ER, energy restriction; GLP-1, glucagon-like peptide 1.
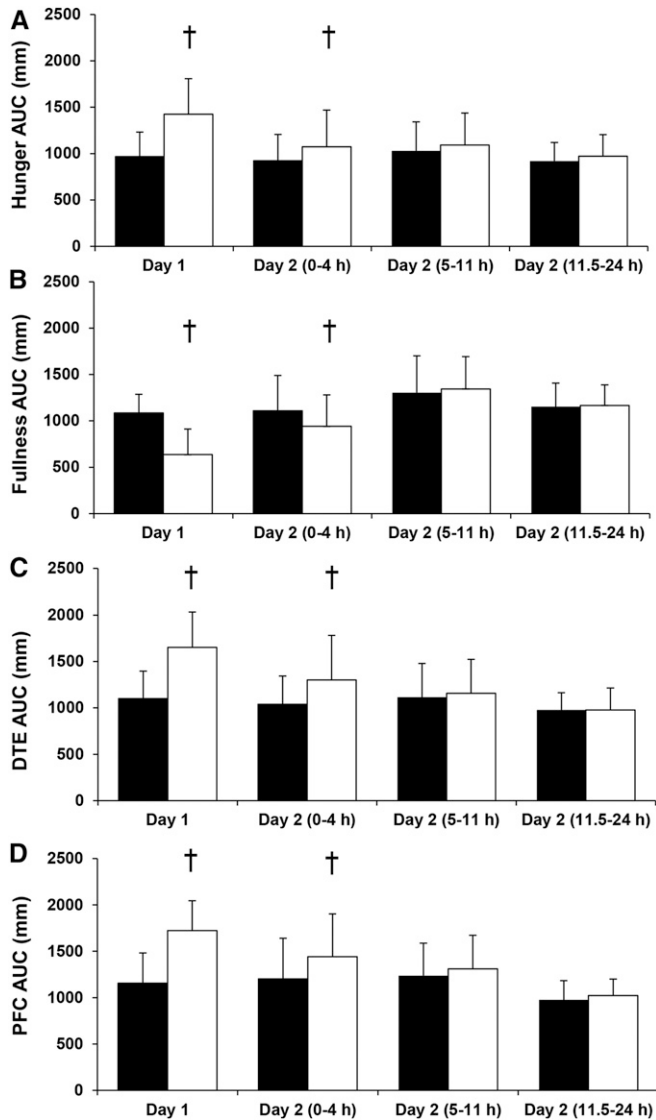
FIGURE 5 AUC for hunger (A), fullness (B), DTE (C), and PFC (D), on day 1 and during the morning (0–4 h), afternoon (5–11 h), and evening (11.5–24 h) of day 2 during the EB trial ■) and ER trial (□). Bars are mean values with vertical error bars representing SDs ($n = 18$). †Significantly different from EB determined by Bonferroni-Holm adjusted paired t test, $P < 0.05$. DTE, desire to eat; EB, energy balance, ER, energy restriction; PFC, prospective food consumption.

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 SER 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 SER did not promote an appetite hormone response indicative of hyperphagia. These results suggest that short periods of SER may reduce energy intake and assist with appetite control in lean men and women.

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 d after an acute (24- to 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-d study was ~20% (2.1 MJ) lower during ER than during EB. Therefore, short-term SER appears to represent a viable strategy for attenuating energy intake in lean men and women.

Subjects reported greater hunger, DTE, and PFC and lower fullness on day 1 during ER than during 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 because 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 d 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 SER. Although counterintuitive, these findings are consistent with a recent study reporting suppressed postprandial acylated ghrelin concentrations after consumption of a diet providing 10% EER for 2 d and including a large component of physical exercise (15). Intralipid infusion has previously been shown 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$_{7-36}$ has been shown to suppress appetite and food intake, suggesting a role in meal termination and postmeal satiety (14). Although GLP-1$_{7-36}$ concentration has been shown to decrease after weight loss (33, 34), 24-h SER did not affect fasting or postprandial GLP-1$_{7-36}$ concentrations in the current study, suggesting this might not be an important regulator of short-term energy balance. GLP-1$_{7-36}$ is also an incretin hormone that responds to ingested nutrients in the stomach and stimulates insulin secretion before 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 SER 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 on release from intestinal L-cells (36). Therefore,

$GLP-1_{7-36}$ could potentially still influence appetite centrally without being detected peripherally.

Although 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 toward obesity, effective methods to assist weight management in lean individuals might be as important as weight loss in overweight and obese individuals. Intermittent SER 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 SER was not indicative of elevated appetite, but paradoxically, subjective appetite was increased and energy intake was $\sim 12\%$ 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 physiologic 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/min) transient reduction in resting energy expenditure was observed during ER, but ER and EB were not significantly different over the assessment period. Although this minor decrement is unlikely to influence energy balance, the laboratory procedures used 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 ($P = 0.06$) during energy restriction, suggesting glycemic control was impaired after 24 h of SER. This has been observed after short periods of complete ER (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 SER. Fasting insulin sensitivity has been shown to improve after 4 mo of intermittent (2 d/wk) SER, but the effect of long-term SER and refeeding cycles on postprandial insulin sensitivity is unknown and warrants further investigation.

In conclusion, 24 h of SER 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 SER and did not respond in a manner indicative of hyperphagia. Therefore, an acute period of SER may assist with energy-balance management in lean men and women. Future studies should aim to examine the chronic effects of intermittent SER on appetite regulation.

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