Anticipation of 24 h severe energy restriction increases energy intake and reduces physical activity energy expenditure in the prior 24 h, in healthy males

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Abstract

Intermittent fasting involves alternating between severely restricted and unrestricted energy intake. Physical activity energy expenditure (PAEE) is reduced during, and energy intake is elevated after, a period of energy restriction, but whether these are altered in anticipation of energy restriction is unknown. The aim of this study was to assess energy intake and PAEE in the 24 h before severe energy restriction. In randomised, counterbalanced order, 14 healthy males completed two 48 h trials over 3 days. On day 1, participants were informed which diet they would receive on day 2; either an energy balanced diet providing 100% (2755 (159) kcal; EB) or an energy restricted diet providing 25% (691 (42) kcal; ER), of their estimated energy requirements. Throughout day 1, *ad-libitum* energy intake was then determined from researcher-provided breakfast (08:30-09:00), lunch (12:30-13:00), afternoon snacks (14:00-18:00) and dinner (19:30-20:00). On day 2, participants consumed their allocated diet as instructed. On day 3, *ad-libitum* energy intake was assessed at breakfast (08:30-09:00). PAEE was measured throughout via integrated heart-rate and accelerometry monitors. Energy intake was 6% greater on day 1 (260 (344) kcal; $P<0.05$) and 14% greater at breakfast on day 3 (223 (59) kcal; $P<0.05$) during ER compared to EB. PAEE was 156 (252) kcal lower on day 1 ($P<0.05$) and 239 (391) lower on day 2 ($P<0.05$) during ER compared to EB. These behavioural compensations meant that the energy deficit produced by 24 h severe energy restriction was attenuated by 1108 (415) kcal (46%) over the study period ($P<0.0001$). These results suggest that compensatory changes in energy intake and PAEE occur before, during and after an acute 24 h period of severe energy restriction, likely lessening the energy deficit created.

Key words: intermittent fasting; energy balance; eating behaviour; physical activity

1. Introduction

Maintenance of a healthy weight is only achieved through careful management of energy balance, with weight gain occurring when energy intake exceeds energy expenditure over a prolonged period of time (Swinburn et al. 2011). This leads to an accumulation of adipose tissue (i.e. overweight/ obesity) and substantially increases the risk of several chronic diseases (Bray et al. 2001). Early to middle adulthood (18-49 years of age) has been identified as a crucial period when the majority of weight gain tends to occur (Ostbye et al. 2011). Although lifestyle modification can achieve weight loss for some people (Greenberg et al. 2009), compensatory alterations in appetite and metabolism favour the regain of lost weight (Polidori et al. 2016), therefore sustaining weight loss in the long-term is notoriously difficult (Anderson et al. 1999). Consequently, it is important to understand how methods of energy restriction affect indices of energy balance, as this will ultimately dictate weight management.

Intermittent fasting is a method of dieting that involves discrete 24-48 h periods of either complete (i.e. by 100%) or severe (by ~75%) energy restriction, separated by periods of *ad-libitum* or adequate energy intake. In contrast to traditional diets, intermittent energy restriction permits periods of unrestricted food intake, with this flexibility suggested to improve diet adherence (Harvie and Howell, 2017). However, by virtue of this flexibility, opportunities are presented where behaviour could be altered to influence the magnitude of the energy deficit that is created. Several studies have demonstrated that a 24-48 h period of complete fasting or severe energy restriction, is not fully compensated for in the subsequent 48-96 h (Clayton et al. 2016a; Clayton et al. 2016b; Levitsky and DeRosimo, 2010; O’Connor et al. 2016; Johnstone et al. 2002). However, energy balance is affected by changes in behaviour before, during and after energy restriction, with all these studies only assessing energy intake *in response* to a period of energy restriction. Meal planning often dictates what and how much we eat in advance of an eating occasion, which is likely to be influenced by how much we expect to want to eat or if we anticipate a future need (Brunstrom, 2011). If expected satiety is reduced in anticipation of a period of energy restriction, this may lead to greater portions being consumed in ‘preparation’, which subsequently compromises the magnitude of energy deficit that is created (Brunstrom et al. 2010; Bell, Roe and Rolls, 2003), something that has recently been reported in the context of exercise (Barutcu et al. 2019). Given the flexibility in dietary behaviour that is permitted by intermittent energy restriction, it is important to determine whether eating behaviour is affected before the period of energy restriction commences.

The majority of nutritional intervention studies focus on energy intake, assuming that a change in energy intake is a surrogate for the change in energy balance. However, physical activity energy expenditure has been shown to be malleable to fasting/ feeding behaviour (Betts et al. 2016). Randomised controlled experiments have shown that extended periods of fasting (implemented by skipping breakfast) reduced spontaneous light-intensity physical activity compared to when a prescribed breakfast was consumed (Betts et al. 2014; Chowdhury et al. 2016), and in these studies the magnitude of this decrease in energy expenditure offset the reduction in energy intake achieved by skipping breakfast. However, it is not known whether consuming a very-low energy diet, rather than implementing a period of complete fasting, has a similar influence on habitual energy expenditure.

The aims of this study were to assess whether a planned period of severe energy restriction (consuming ~25% of estimated energy requirements (EER)) affected participants energy intake, physical activity energy expenditure and subjective appetite during the prior 24 h, and whether habitual physical activity energy expenditure was affected during a 24 h period of severe energy restriction.

2. Methods

2.1.Participants

Fourteen healthy males provided written consent and completed the study (**Table 1**). Participants were not restrained, disinhibited or hungry eaters (Three Factor Eating Questionnaire; Stunkard and Messick, 1985). All participants were active, non-smokers, weight stable for 6 months (self-reported), not currently dieting, and were not consuming any medication known to affect appetite. The study was approved by the Nottingham Trent University Human Invasive Ethics Committee (Ref: 526).

Table 1. Participant baseline characteristics. Values are means (SD).

Characteristic	Participants (n=14)
Sex	Male
Race	Caucasian n=14
Age (y)	23 (5)
Weight (kg)	81.76 (7.98)
Height (m)	1.82 (0.07)
BMI (kg·m^{-2})	24.59 (2.01)
Body fat (%)	16.51 (3.95)
Dietary restraint[1]	6 (2)
Dietary disinhibition[1]	9 (4)
Hunger[1]	7 (2)
Resting metabolic rate (kcal)[2]	1841 (114)

[1] Three factor eating questionnaire (Stunkard and Messick, 1985)
[2] Estiamted via predictive equation (Mifflin et al. 1990)

2.2. Study design

Participants completed a 1-day preliminary trial and two experimental trials. Experimental trials were conducted over a 48 h period (from 08:30 on day 1 until 08:30 on day 3) and were administered in a randomised, counterbalanced order. On day 1, all participants food intake was measured, after they were informed which of the two diets they would receive the following day. On day 2, participants consumed a pre-prepared 24 h standardised diet, providing either 100% (EB) or 25% (ER) of EER. On day 3, food intake was measured at an *ad-libitum* buffet meal. Physical activity was measured continuously throughout each trial.

2.3. Preliminary trial and standardisation procedures

During the preliminary trial, participants body mass (Adam CFW150; Adam Equipment Limited; Milton Keynes; UK), height (Seca; Hamberg; Germany) and skin-fold thickness (bicep, tricep, subscapular, iliac crest; Harpenden, West Sussex, UK) were measured, with BMI and body fat percentage (Durnin and Womersley, 1974) calculated. This was followed by an incremental ambulatory exercise test, with participants completing 3-minute stages, at 3.2 km/h, 5.2 km/h, 5.6 km/h at 10% gradient and 9 km/h (Templeman et al. 2018; Brage et al. 2007). In the final minute of each stage, mean heart rate was recorded (Polar H10, Polar, Warwick, UK) and expired gas was collected into a Douglas bag. Expired gas was analysed for oxygen and carbon dioxide concentration (MiniMP 5200, Servomex, East Sussex, UK), volume (Dry gas meter, Cranlea, Birmingham, UK) and temperature (Digital thermometer, Fisher Scientific Ltd, Loughborough, UK), with energy expenditure calculated via indirect calorimetry (Frayn, 1983). This data was then used to individually calibrate physical activity monitors (Actiheart, CamNtech, Cambridge, UK). Participants were also fully familiarised with all *ad-libitum* buffet procedures during this preliminary trial.

Twenty-four hours before the first experimental trial, participants recorded their dietary intake and physical activity. This was then replicated in the 24 h preceding the second trial. Participants were asked to avoid any unnecessary activity, with alcohol and strenuous exercise strictly prohibited in the 24 h pre-trial and 48 h trial period. Participants completed each trial on the same days of the week, with no longer than two weeks between experimental trials.

2.4. Protocol

For each trial, participants attended the laboratory on three consecutive mornings at 08:00 after a ≥10 h overnight fast, with body mass (in minimal clothing) and capillary blood glucose concentration (Biosin C-Line; EKF Diagnostics; Cardiff; UK) measured 20 minutes after arrival. Changes in body mass were used to as a surrogate marker of adherence to the study protocol.

After baseline measures on day 1, an Actiheart monitor was fitted and participants completed an appetite questionnaire. Participants were then informed of the diet they would receive on day 2. For the EB trial, participants were told: *"Tomorrow you will consume a diet providing 100% of your energy (calorie) requirements. This will contain [participants standardised diet energy content to maintain energy balance rounded to nearest 10 kcal] and will be a similar amount of food to what you would normally eat"*. For the ER trial, participants were told: *"Tomorrow you will consume a diet providing 25% of your energy (calorie) requirements. This will contain [participants standardised diet energy content to provide 25% of energy requirements rounded to nearest 10 kcal] and will be about one quarter of the amount of food you would normally eat"*. After a 5-minute interval, participants completed another appetite questionnaire, immediately followed by an *ad-libitum* breakfast (~08:30-09:00). Participants left the laboratory after breakfast and returned to the laboratory for an *ad-libitum* lunch (~12:30-13:00). After lunch, participants were provided with a selection of snacks that could be consumed *ad-libitum* 14:00-18:00 and an *ad-libitum* pasta-based evening meal to be consumed at home 19:30-20:00. *Ad-libitum* water intake was permitted throughout the day and was recorded.

Participants returned to the laboratory on day 2 and were provided with a standardised diet providing either 100% (EB) or 25% (ER) of EER. Breakfast was consumed in the laboratory (08:30), after which participants left with all remaining food and drink items for the day, along with instructions of when to consume each item. On day 3, participants returned to the laboratory and baseline measures were repeated, after which an *ad-libitum* breakfast meal (identical to day 1) was provided (08:00-08:30).

2.5. Standardised diets

Diets provided on day 2 were tailored to individual energy requirements and food preferences to encourage adherence. Resting metabolic rate was estimated for each participant using a predictive equation (Mifflin et al. 1990) and multiplied by a physical activity level of 1.5 (indicating light activity), determining EER. During EB, 100% of EER was provided as four meals: 20% (of total food energy) at 08:30 (cereal, milk, orange juice and apple), 30% at 12:30 (white bread, mayonnaise, chicken, salad and cookies), 10% at 16:00 (yogurt and cereal bar) and 40% at 19:30 (pasta, Bolognese sauce, chicken, cookies). During ER, 25% of EER was split into three meals: 7% (of total food energy) at 08:30 (apple), 32% at 12:30 (chicken and salad) and 61% at 19:30 (pasta, Bolognese sauce, chicken). Water was also provided with breakfast during ER, which was of the same volume as the breakfast provided on EB. Additional water was prescribed at 35 $mL \cdot kg^{-1}$ body mass (2400 (260) mL) on both trials and was evenly distributed throughout the day. Similar foods were provided on both trials, with the ER diet created by removing or reducing high fat and high carbohydrate foods from the EB diet, as described previously (Clayton et al. 2016a).

2.6. Ad-libitum food and water intake

Energy and macronutrient intake was assessed at a multi-item breakfast (08:30-09:00), a multi-item lunch (12:30-13:00), optional snacks (14:00-18:00) and a homogenous dinner (19:30-20:00) on day 1, as well as a multi-item breakfast on day 3 (08:30-09:00). Amounts consumed at each meal were quantified by weighing each food item before and after consumption, with energy and macronutrient intake ascertained from manufacturer values. Breakfast and lunch meals were served in the laboratory in an isolated feeding booth with no interaction between participants and investigators. Food was provided in excess of expected consumption, with more food available on request. Participants were given 30 minutes to eat each meal, and were explicitly instructed to eat until they felt *"comfortably full and satisfied"*. Items provided for each *ad-libitum* eating occasion are detailed in **Table 2.** The dinner meal was a homogenous main meal consisting of pasta, Bolognese sauce and olive oil, with chocolate-chip cookies for dessert, which participants consumed at home. The main meal was prepared the day prior to trials using identical cooking and cooling procedures, and was provided in a large plastic container. Participants were required to select a portion and warm it before eating. Participants were asked to eat this meal from the same plate or bowl during both trials, which they could refill as often as desired within 30 minutes, and they were asked to eat until they felt *"comfortably full and satisfied"*. Chocolate-chip cookies were provided in the same container and in the same quantity for both trials. All items consumed outside the laboratory were weighed before being provided and reweighed upon return to the laboratory on day 3 of the trial. Water intake was permitted *ad-libitum* and was recorded.

Table 2: Food items provided during each *ad-libitum* eating occasion.

Item	**Energy density (kcal·100g^{-1})**	**Approx. amount provided (g)**	**Item**	**Energy density (kcal·100g^{-1})**	**Approx. amount provided (g)**
Breakfast items (08:30-09:00)					
White bread	238	800	Light spread	398	500
Brown bread	233	800	Sliced ham	107	200
Coco Pops cereal	382	480	Grated cheese	416	50
Rice Krispies cereal	387	510	Yogurt	90	375
Muesli	369	550	Sugar	400	500
Weetabix	362	340	Apple	53	250
Semi-skimmed milk	50	2000	Clementine	47	200
Jam	244	300	Orange Juice	40	1000
Marmalade	257	450	Sugar free squash	20	1000
Nutella	539	400	Water	0	1000
Lunch items (12:30-13:00)					
White bread	238	800	Yogurt	90	375
Brown bread	233	800	Crisps	256	50
Tomato	20	150	Chocolate chip cookies	491	200
Cucumber	16	200	Apple	53	250
Light mayonnaise	264	430	Clementine	47	200
Sliced chicken	101	200	Orange Juice	40	1000
Sliced ham	107	200	Sugar free squash	20	1000
Cheese	416	50	Water	0	1000
Light spread	398	500			
Snack items (14:00-18:00)					
Fun size Mars bar	443	40	Apple	53	250
Fun size Twix bar	495	40	Clementine	47	200
Special K cereal bar	384	55	Crisps	256	50
Dinner items (19:30-20:00)					
Pasta (cooked)	176	1100	Olive oil	900	32
Bolognese sauce	45	400	Chocolate chip cookies	491	200

2.7. Energy expenditure

Energy expenditure was assessed in 15 second epochs from 08:30 on day 1 until 08:30 on day 3 via an Actiheart monitor, which integrates heart rate and accelerometry to yield the most accurate estimation of physical activity energy expenditure of any wearable device (Chowdhury et al. 2017). Dietary induced thermogenesis was estimated from participants macronutrient intake during trials (Westerterp, 2004), then added to physical activity energy expenditure derived from the Actiheart to summate total non-resting energy expenditure. To improve the validity of the energy expenditure estimation, monitors were individually calibrated using the heart rate-energy expenditure regression equation from the sub-maximal ambulatory test conducted during the preliminary trial (Brage et al. 2007). Data was considered valid if less than 10% of the activity trace was 'lost' during waking hours, and <30% of the heart rate trace was 'interpolated' by the software (Edinburgh et al. 2019). All Actiheart data collected in the present study met this criteria, so all data were included in analysis.

2.8. Subjective appetite sensations

Hunger, fullness, desire to eat (DTE), prospective food consumption (PFC) and nausea were assessed via a questionnaire, immediately before and after each meal (excluding snacks). An additional questionnaire was provided before and 5 minutes after participants were informed which trial they were completing on day 1. Ratings were provided on a 100 mm visual analogue scales with anchors of "not at all/ none at all/ no desire at all" and "extremely/ a lot" placed at 0 and 100 mm, respectively.

2.9. Statistical analysis

Data were analysed using SPSS 24.0 (IBM, Chicago, USA). All data was checked for normality using a Shapiro-Wilk test. Energy intake, macronutrient intake and PAEE data were analysed as a total for each trial and as a sub-total for each day of the study separately. Energy and macronutrient intake was also analysed at each individual *ad-libitum* eating occasion. In each case, data were expressed as a single value for each trial and analysed using a paired samples *t*-test (normally distributed data) or Wilcoxon-Signed Rank test (non-normally distributed data), as appropriate. PAEE data were also sub-divided and analysed by times-of-day, i.e. early morning (06:00-08:59), morning (09:00-11:59), afternoon (12:00-16:59), evening (17:00-21:59) and overnight (22:00-05:59), and by accepted thresholds for intensity (Haskell et al. 2007), i.e. sedentary (<1.5 METS), light (1.5-2.9 METS), moderate (3-5.9 METS) and vigorous (>6 METS). Similarly, data for each sub-division were expressed as a single value for each and analysed using a paired samples *t*-test or Wilcoxon Signed-Rank test, as appropriate. Repeated measures ANOVA were used to evaluate main effects of time, trial and time-by-trial interactions for variables with multiple time points (e.g. hunger, fullness, desire to eat, prospective food consumption, blood glucose and body mass). Where interaction effects were observed, Holm-Bonferroni adjusted *post-hoc* paired *t*-tests or Wilcoxon Signed-Ranks tests were conducted. For appetite-related variables, area under the curve (AUC) was calculated using the trapezoidal method, and were analysed using a *t*-test or Wilcoxon Signed-Rank test, as appropriate. Data sets were determined to be statistically significantly different when $P<0.05$. Data are presented as mean (SD) in text and tables and as mean (SEM) in figures.

3. Results

3.1. Energy and macronutrient intake

There was no difference in energy intake at any discrete meal on day 1 (breakfast: *P*=0.235; lunch: *P*=0.380; snack: *P*=0.203; dinner: *P*=0.767; **Figure 1**), but total *ad-libitum* energy intake on day 1 was 6% greater during ER compared to EB (260 (344) kcal; *P*<0.05; **Figure 1**). Greater total energy intake on ER was driven by greater carbohydrate intake (*P*<0.05), as well as a trend for greater protein intake (*P*=0.083), with no differences in fat (*P*=0.138) or fibre (*P*=0.584) intake. Water intake was also greater on ER compared to EB (*P*<0.01; **Table 3**).

On day 2, when each participant's food intake was prescribed and provided, energy intake was 2065 (118) kcal lower on ER compared to EB (**Figure 1**).

At breakfast on day 3, *ad-libitum* energy intake was 17% greater (176 (226) kcal) during ER compared to EB (*P*<0.05). This was again driven by greater carbohydrate intake during ER (*P*<0.001), with no differences in protein (*P*=0.141), fat (*P*=0.179) or fibre (*P*=0.885) intake between trials. Water intake tended to be greater on ER compared to EB (*P*=0.067; **Table 3**). When comparing *ad-libitum* energy intake between the identical breakfast meals provided on day 1 and 3, there was a time (*P*<0.01), a trial (*P*<0.05) but no interaction effect (*P*=0.352) identified. Across both trials, a*d-libitum* energy intake was 16% greater during day 3 compared to day 1 (163 (227) kcal; *P*<0.001). Energy intake was also 16% (135 (254) kcal) greater on day 1 and 3 combined during ER, compared to EB (*P*<0.01).

Over the study period, *ad-libitum* energy intake was 436 (463) kcal (8%) greater during ER compared to EB (*P*<0.01), which was sufficient to replace 21% of the energy deficit created on day 2. Including day 2, total energy intake over the 3-day study period was 1629 (423) kcal lower during ER compared EB (EB: 8321 (1622); ER: 6692 (1739) kcal; *P*<0.001).

[Figure 1 here]

Table 3: Energy and macronutrient intake on each day of the study period

	Day 1		Day 2		Day 3 (breakfast only)	
	EB	**ER**	**EB**	**ER**	**EB**	**ER**
Energy (kcal)	4322 (1065)	4582 (1193)†	2755 (159)	691 (42)†	1244 (567)	1420 (596)†
Protein (g)	140 (35)	148 (41)	126 (8)	80 (5)†	48 (23)	53 (21)
CHO (g)	588 (143)	626 (149)†	367 (23)	69 (4)†	187 (88)	215 (95)†
Fat (g)	148 (49)	156 (53)	84 (6)	10 (1)†	31 (17)	36 (19)
Fibre (g)	41 (12)	42 (10)	15 (2)	5 (0)†	12 (6)	13 (4)
Water (g)	4568 (839)	5217 (1241)†	3488 (304)	3215 (295)†	974 (276)	1064 (336)

Values are means (SD). EB: energy balance trial; ER: energy restriction trial. CHO; carbohydrate. † indicates significantly different from EB ($P<0.05$).

3.2. Energy expenditure

PAEE was 11% lower on day 1 (1221 (474) vs. 1064 (436) kcal; $P<0.05$) and 18% lower on day 2 (1183 (409) vs. 944 (370) kcal; $P<0.05$) during ER compared to EB (**Figure 2**). Over the study period, PAEE was 16% lower during ER compared to EB (2403 (700) vs. 2008 (692) kcal; *P*<0.01).

When analysed by time of day, PAEE was significantly lower in the afternoon on day 2 during ER compared to EB (355 (110) vs. 207 (118) kcal; *P*<0.001), and tended to be lower in the afternoon on day 1 during ER (*P*=0.078). There was also a tendency for lower PAEE overnight during ER on day 2 (*P*=0.084). No other time-period differed significantly between trials (*P*>0.230; **Figure 2**). When separated by intensity, analysis revealed participants engaged in less light intensity PAEE during across the total study period during ER (*P*<0.001), with light intensity PAEE lower during ER on both day 1 (*P*<0.05) and day 2 (*P*<0.01), and vigorous intensity PAEE tending to be lower on day 1 (*P*=0.084). There were no further differences between trials for PAEE intensity (*P*>0.114; **Figure 2**).

Using established constants for the thermogenic effect that each macronutrient has upon ingestion (Westerterp, 2004), dietary induced thermogenesis was estimated to be greater during EB on day 2 (248 (16) vs. 102 (7) kcal; *P*<0.001), and slightly greater during ER on day 1 (340 (75) vs. 361 (89) kcal; *P*<0.05).

Over the study period, PAEE was 395 (452) kcal lower during ER compared to EB (*P*<0.01), which was sufficient to replace 19% of the energy deficit created by the energy restriction intervention on day 2. Accounting for differences in dietary induced thermogenesis between trials, energy expenditure was 521 (469) kcal lower during ER (*P*<0.001), compensating for 25% of dietary induced energy deficit achieved on day 2.

[Figure 2 here]

3.3. Subjective appetite sensations

There were trial (*P*<0.001), time (*P*<0.001) and interaction (*P*<0.001) effects for hunger, fullness, DTE and PFC. There were no trial (*P*=0.334), time (*P*=0418) or interaction (*P*=0.393) effects for nausea. On day 1, there was a tendency for DTE to be lower before lunch (*P*=0.059) and fullness was greater after dinner and before bed (*P*<0.05) during ER compared to EB. Informing participants that they were completing the ER trial did not immediately influence fullness, DTE or PFC (*P*>0.403), but tended to increase hunger (*P*=0.088), and there was no immediate effect on any marker of appetite when they were told they were completing the EB trial (*P*>0.276). AUC over the entire day was greater for DTE during EB compared to ER (*P*<0.05), but there was no difference in AUC for hunger (*P*=0.370), fullness (*P*=0.205), PFC (*P*=0.594) or nausea (*P*=0.791; **Figure 3**).

On day 2, there was no difference in any subjective appetite measure before breakfast (*P*>0.119). After breakfast, hunger and DTE were greater (*P*<0.01), PFC tended to be greater (*P*=0.062), and fullness was lower (*P*<0.05) during ER. Before lunch, DTE was greater (*P*<0.05) and PFC tended to be greater (*P*=0.064) during ER. Hunger, DTE and PFC were greater, with fullness lower (*P*<0.05), after lunch during ER. There were no differences in any appetite measure before dinner (*P*>0.168), but hunger, DTE and PFC were greater, and fullness lower (*P*<0.001), after dinner during ER. Hunger, DTE and PFC were greater, and fullness lower (*P*<0.05), before bed during ER. AUC for the whole of day 2 was greater during ER for hunger, DTE and PFC, and lower for fullness, compared to EB (all *P*<0.001), but there was no difference in nausea (*P*=0.845; **Figure 3**).

On day 3, PFC was greater and fullness lower (*P*<0.05) before breakfast, with no difference in any appetite measure after breakfast (*P*>0.244).

[Figure 3 here]

3.4. Body mass and blood glucose concentration

There were time (*P*<0.001) and interaction (*P*<0.001) effects, but no effect of trial (*P*=0.713) for body mass. Body mass on day 3 was 0.7 (0.7) kg lower on ER compared to EB (*P*<0.01; **Table 4**). Between day 2 and day 3, body mass decreased during ER (*P*<0.001) and tended to decrease during EB (*P*=0.094). The amount of body mass lost between day 2 and 3 was considerably greater during ER compared to EB (1.4 (0.7) kg vs. 0.7 (0.7) kg; *P*<0001). Body mass also increased by 0.5 (0.7) kg between day 1 and 2 during ER (*P*<0.05).

There were no main time (*P*=0.293), trial (*P*=0.564) or interaction (*P*=0.054) effects for blood glucose concentration.

Table 4: Morning body mass and blood glucose measurements during each day of each experimental trial

	Energy Balance (EB)			Energy Restriction (ER)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Body mass (kg)	81.35 (8.24)	81.66 (8.36)	81.26 (8.43)	81.51 (8.32)	81.99 (8.78)*	80.59 (8.33)‡†
Blood glucose (mmol·L^{-1})	4.38 (0.41)	4.51 (0.29)	4.45 (0.27)	4.50 (0.37)	4.43 (0.47)	4.28 (0.30)

Values are means (1SD). † indicates significant difference to EB at corresponding time point; *indicates significant difference to day 1 during same trial (*P*<0.05); ‡ indicates significant difference to day 2 during the same trial.

4. Discussion

The primary aim of this study was to investigate whether compensatory alterations in indices of energy balance occur in anticipation of an acute period of severe energy restriction. The study found that energy intake is increased 6% and physical activity energy expenditure (PAEE) decreased 11%, in the 24 h preceding an acute 24 h period of energy restriction (ER), compared to an energy balance (EB) control trial. Furthermore, PAEE decreased 18% during the 24 h period of severe energy restriction. These results indicate that compensatory behavioural alterations, on both sides of the energy balance equation, occur in anticipation and in response to a dietary induced energy deficit.

Previous studies have been designed to assess how appetite and energy intake responded after a period of severe energy restriction (consuming 25% of EER). These studies have consistently reported, as expected, that appetite and energy intake increases following a 24-48 h period of severe energy restriction, compared to an adequate energy control trial in the short term (Clayton et al. 2016a; Clayton et al. 2016b; O'Connor et al. 2016; Johnstone et al. 2002). However, the absolute increase in energy intake observed in response to severe energy restriction is small compared to the energy deficit created by the period of energy restriction, and as such, relative energy intake is consistently reported to be lower during severe energy restriction (Clayton et al. 2016a; Clayton et al. 2016b; O'Connor et al. 2016; Antoni et al. 2016).

The results of the present study indicate that knowledge of a future period of severe energy restriction, as would be the case in a real-world setting, results in an anticipatory increase in energy intake. In the present study, participants increased their energy intake by ~260 kcal on day 1, essentially compensating for ~12% of the energy deficit, before even undertaking the 24 h period of severe energy restriction on day 2. This data has implications for intermittent fasting diets that involve alternating between periods of severely restricted food intake and periods of *ad-libitum* food intake. A popular variation of intermittent fasting is the *5:2 diet*, which typically involves splitting the seven-day week into two days of severely restricted food intake (~500 kcal) and five days of unrestricted eating (Harvie and Howell, 2017). The day-to-day flexibility of this method is thought to be one of the key reasons for its popularity (Harvie and Howell, 2017), but consequently, this presents opportunities for individuals to increase energy intake above their adequate energy requirements outside of the defined period of restriction. Previous studies have demonstrated that compensatory eating occurs *after* a period of severe energy restriction, but the present study provides novel findings that compensatory eating also occurs *before* a period of severe energy restriction has commenced. This is likely to reduce the magnitude of the energy deficit achieved.

Appetite is thought to be governed by homeostatic, environmental and cognitive factors that culminate in the initiation and termination of an eating episode. It is therefore interesting to note that, despite consuming more food, participants did not report any orexigenic differences in appetite sensations during day 1 of the ER trial, in the present study. Indeed, the only observed difference in appetite on day 1 was a reduced desire to eat during the ER trial, which likely reflects the fact that participants consumed more food during the ER trial. This difference in desire to eat may have also been magnified by the fact that appetite was only assessed before and after each meal. The energy intake results align closely with an alternative theory on eating behaviour, termed ‘expected satiety’, in that meal size is determined in advance of an eating occasion (Brunstrom, 2011). In the context of the present study, participant’s expectations on how satiated (or hungry) they would feel on day 2 may have influenced their eating behaviour on day 1. Recently, Potter et al. (2019) found that individuals who were not successful with intermittent fasting reported that they were more likely to eat in anticipation of a future need, compared to individuals currently undertaking intermittent fasting. The participants of the present study were healthy males not currently undertaking any weight management programme and were not accustomed to intermittent fasting diets. It would be interesting to determine whether eating behaviour differed after repeated exposure to periods of severe energy restriction, particularly as expected satiety is modulated by previous experience, which may influence portion size selection (Brunstrom et al. 2008).

The present study also observed a decrease in PAEE in the 24 h before, and during, the period of severe energy restriction. In essence, this reduction in PAEE served to reduce the energy deficit achieved by the dietary energy restriction study intervention on day 2 by ~400 kcal (16%), compared to the energy balanced control trial. The attenuation in PAEE during the period of severe energy restriction may be the result of perceived lethargy or reduced substrate availability, resulting in either the conscious or subconscious reduction of non-essential physical activity (Betts et al. 2016). Previous studies have reported a similar reduction in PAEE in response to extended morning fasting (Betts et al. 2014; Chowdhury et al. 2016). In one study, average daily PAEE was ~440 kcal greater in lean individuals who consumed a 700 kcal breakfast for six weeks, compared to individuals who skipped breakfast and fasted until midday (Betts et al. 2014). Of note, a significant proportion of this difference (~180 kcal) occurred before midday, coinciding with the time when no energy was consumed in the breakfast skipping group (Betts et al. 2014). The current study provides an important addition to the literature, as the reduction in PAEE detected using combined heart-rate accelerometers (i.e. Actiheart monitors) on day 1 cannot be attributed to a fasting-related reduction in heart rate (Matsumoto et al. 2001), as could be suggested with previous studies. Therefore, in conjunction with previous studies, these findings provide strong evidence that complete or severe energy restriction leads to a conscious or subconscious concurrent reduction in PAEE. In the context of obesity, it should be noted that 10% weight gain achieved by over-nutrition was associated with an increase in energy expenditure, which could not be fully explained by an increase in

RMR, suggesting that PAEE may also increase (although likely to a lesser extent) in the presence of an energy surplus (Leibel et al. 1995).

To our knowledge, this is the first study to observe a reduction in PAEE in anticipation of a period of severe energy restriction in humans. These findings indicate that PAEE is not solely affected by concurrent nutrient availability, but also regulated in response to a threat to energy homeostasis. This may stem from an evolutionary trait in humans to conserve energy in preparation for periods of reduced food availability (Leiberman, 2006). In the wild, when food availability is intermittent, some animals will moderate their non-essential thermogenesis, enabling their endogenous energy reserves to sustain them for the longest time possible (Halsey, 2016). In addition, animals that gorge on food when there is an abundance, will increase their energy expenditure as a means of maintaining a stable body weight (Halsey, 2018). These examples highlight that sustaining a healthy body weight is of critical importance for wild animals, likely because a fluctuation may make them vulnerable to predators or reduce reproductive proficiency (Halsey, 2016). These are generally not concerns shared by humans in the modern world, but it is well-established that the appetite regulatory system is sensitive to an energy deficit, but less so to an energy surplus (Rogers and Brunstrom, 2016), suggesting a disproportionate response in humans which favours weight gain. An interesting extension to this work would be to consider participants subjective psychological responses to energy restriction, which would help to determine the extent to which PAEE is consciously altered.

Linked to this, one study reported that misleading participants to believe that they would not be eating breakfast resulted in an increase in fasting concentrations of the orexigneic hormone ghrelin, which remained elevated post-prandially even after participants had consumed breakfast (Ott et al. 2012). Ghrelin has also been suggested to be involved in the regulation of physical activity via the hypothalamic neuropeptide AgRP (Pfluger et al. 2011), suggesting there is interplay between mechanisms affecting components of energy balance. Whilst beyond the scope of this study, alterations in appetite hormone concentrations occurring after participants were informed of their day-2 diet may lead to reduced physical activity via this pathway, although future studies would be required to elucidate a mechanistic link between anticipatory appetite and physical activity.

Long-term studies have shown that intermittent energy restriction can be successful for achieving weight loss of 5-8% over 12-24 weeks (Harvie and Howell, 2017). However, it is important to note that these studies often include nutritionist support and sometimes provide meals for participants, typically services not available to the wider public (Gibson and Sainsbury, 2017). The flexibility permitted by intermittent fasting is considered a major appeal of the diet, as it negates arduous calorie counting by interspersing 24 h periods of severe energy restriction around periods of unrestricted eating. By design, an intermittent diet provides opportunities for compensatory behaviours to reduce the magnitude of the energy deficit created during the periods of severe energy restriction. Data from the current study indicates that increases in energy intake and reductions in PAEE attenuate this energy deficit considerably, rendering efforts to severely restrict energy intake on certain days less effective than might be assumed.

The current study found an increase in food intake with a concurrent reduction in physical activity in *anticipation* of severe energy restriction (by ~390 kcal), a reduction in physical activity *during* the period of energy restriction (by ~435 kcal) and an increase in energy intake at the first meal following the period of energy restriction (by ~175 kcal). In total, these compensatory changes accounted for 1108 (415) kcal of the 2065 (118) kcal reduction in energy intake achieved by the severe energy restriction intervention on day 2. Considering also that other similarly designed studies have observed further compensations in energy intake, in the 48h following a period of severe energy restriction (Clayton et al. 2016; Clayton et al. 2016), it is clear that the magnitude of the energy deficit achieved by severe energy restriction would be reduced considerably by compensatory behavioural alterations. Physical activity and ‘binge’ eating are also associated with chronic disease (Roberts and Bernard, 2005; Parry et al. 2017), therefore, even if the energy deficit conserved after compensation is still sufficient to prevent weight gain, future studies will need to determine the impact of these behaviours on long-term metabolic health.

The findings of the present study provide novel insight into the regulation of energy balance in anticipation of energy restriction, but it is not without limitations. Firstly, this study was conducted in healthy male participants, and therefore the results cannot necessarily be extrapolated to other population groups, specifically overweight or obese individuals. Secondly, Potter et al. (2019) reported that beliefs about the effectiveness or difficulty of the intermittent fasting diets were key factors in determining adherence and success. Therefore, it is likely the participants in this study were not sufficiently motivated to preserve the energy deficit achieved by severe energy restriction on day 2 of the study, or were not sufficiently experienced with the diet to know how they would be affected. This may have influenced energy balance through an increase in energy intake, although this is unlikely to have affected energy expenditure. Thirdly, this study only investigated a single exposure to severe energy restriction, so it is not known whether the observed behavioural changes persist after multiple exposures. Finally, despite being an accurate way to quantify energy intake, the buffet meal context used in this study is unlikely to reflect reality for individuals undertaking intermittent fasting habitually.

In conclusion, the current study has shown that compensatory changes in physical activity energy expenditure and energy intake may occur before, during and after a period of severe energy restriction, and these changes serve to reduce the magnitude of the energy deficit that is achieved by severe energy restriction. These results suggest that flexible intermittent diets that incorporate severe energy restriction interspersed with periods of unrestricted intake may induce a smaller energy deficit than anticipated, which may have implications for long-term weight management. Future studies should aim to develop strategies to mitigate against energy compensation during intermittent dieting, with the current study indicating these strategies should be implemented before and after periods of energy restriction, and target both sides of the energy balance paradigm.

Declaration of competing interests

None

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Author contributions

DJC, LJJ and RJ designed the study. RJ and DJC performed data collection and analysis. DJC and RJ wrote the manuscript with assistance from LJJ. All authors approved the final version of the manuscript.

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Figure Captions

Figure 1: Energy intake for day 1 (left), day 2 (centre) and day 3 (right), during the energy balance (EB) and energy restriction (ER) trials. Total energy intake on each day is sub-divided by meal – breakfast (black bar), lunch (grey bar), snack (white bar) and dinner (crosshatch bar). † indicates a significant difference in total energy intake between ER and EB during the corresponding day ($P<0.05$). P-values between the bars represent the comparison between trials at each meal during the corresponding day. Values are mean (SEM).

Figure 2: Physical activity energy expenditure during day 1 (left) and day 2 (right) during the energy balance (EB) and energy restriction (ER) trials. Total energy expenditure is sub-divided by activity intensity (a) – sedentary (black bar), light (grey bar), moderate (white bar) and vigorous (crosshatch bar) and by time of day (b) – early morning (06:00-08:59), morning (09:00-11:59), afternoon (12:00-16:59), evening (17:00-21:59) and overnight (22:00-05:59). † indicates a significant difference in total physical activity energy expenditure between ER and EB during the corresponding day ($P<0.05$). P-values between the bars represent the comparison between trials for energy expenditure at the each intensity/time of day during the corresponding day. Values are mean (SEM).

Figure 3: Hunger (a), fullness (b), desire to eat (DTE) (c) and prospective food consumption (PFC) (d) during the energy balance (EB; black squares; black bar) and energy restriction (ER; white circle; white bar) trials. Data is presented at each time point (left) and as a time-averaged area under the curve for each day (right). Values are mean (SEM). † indicates significant difference to EB at corresponding time point.