

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

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5 **Abstract**

6 Intermittent fasting involves alternating between severely restricted and unrestricted energy  
7 intake. Physical activity energy expenditure (PAEE) is reduced during, and energy intake is  
8 elevated after, a period of energy restriction, but whether these are altered in anticipation of  
9 energy restriction is unknown. The aim of this study was to assess energy intake and PAEE in  
10 the 24 h before severe energy restriction. In randomised, counterbalanced order, 14 healthy  
11 males completed two 48 h trials over 3 days. On day 1, participants were informed which diet  
12 they would receive on day 2; either an energy balanced diet providing 100% (2755 (159) kcal;  
13 EB) or an energy restricted diet providing 25% (691 (42) kcal; ER), of their estimated energy  
14 requirements. Throughout day 1, *ad-libitum* energy intake was then determined from  
15 researcher-provided breakfast (08:30-09:00), lunch (12:30-13:00), afternoon snacks (14:00-  
16 18:00) and dinner (19:30-20:00). On day 2, participants consumed their allocated diet as  
17 instructed. On day 3, *ad-libitum* energy intake was assessed at breakfast (08:30-09:00). PAEE  
18 was measured throughout via integrated heart-rate and accelerometry monitors. Energy intake  
19 was 6% greater on day 1 (260 (344) kcal;  $P<0.05$ ) and 14% greater at breakfast on day 3 (223  
20 (59) kcal;  $P<0.05$ ) during ER compared to EB. PAEE was 156 (252) kcal lower on day 1  
21 ( $P<0.05$ ) and 239 (391) lower on day 2 ( $P<0.05$ ) during ER compared to EB. These behavioural  
22 compensations meant that the energy deficit produced by 24 h severe energy restriction was  
23 attenuated by 1108 (415) kcal (46%) over the study period ( $P<0.0001$ ). These results suggest  
24 that compensatory changes in energy intake and PAEE occur before, during and after an acute  
25 24 h period of severe energy restriction, likely lessening the energy deficit created.

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

27

## 1. Introduction

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

39 Intermittent fasting is a method of dieting that involves discrete 24-48 h periods of either  
40 complete (i.e. by 100%) or severe (by ~75%) energy restriction, separated by periods of *ad-*  
41 *libitum* or adequate energy intake. In contrast to traditional diets, intermittent energy restriction  
42 permits periods of unrestricted food intake, with this flexibility suggested to improve diet  
43 adherence (Harvie and Howell, 2017). However, by virtue of this flexibility, opportunities are  
44 presented where behaviour could be altered to influence the magnitude of the energy deficit  
45 that is created. Several studies have demonstrated that a 24-48 h period of complete fasting or  
46 severe energy restriction, is not fully compensated for in the subsequent 48-96 h (Clayton et al.  
47 2016a; Clayton et al. 2016b; Levitsky and DeRosimo, 2010; O'Connor et al. 2016; Johnstone  
48 et al. 2002). However, energy balance is affected by changes in behaviour before, during and  
49 after energy restriction, with all these studies only assessing energy intake *in response* to a  
50 period of energy restriction. Meal planning often dictates what and how much we eat in advance  
51 of an eating occasion, which is likely to be influenced by how much we expect to want to eat

52 or if we anticipate a future need (Brunstrom, 2011). If expected satiety is reduced in  
53 anticipation of a period of energy restriction, this may lead to greater portions being consumed  
54 in ‘preparation’, which subsequently compromises the magnitude of energy deficit that is  
55 created (Brunstrom et al. 2010; Bell, Roe and Rolls, 2003), something that has recently been  
56 reported in the context of exercise (Barutcu et al. 2019). Given the flexibility in dietary  
57 behaviour that is permitted by intermittent energy restriction, it is important to determine  
58 whether eating behaviour is affected before the period of energy restriction commences.

59 The majority of nutritional intervention studies focus on energy intake, assuming that a change  
60 in energy intake is a surrogate for the change in energy balance. However, physical activity  
61 energy expenditure has been shown to be malleable to fasting/ feeding behaviour (Betts et al.  
62 2016). Randomised controlled experiments have shown that extended periods of fasting  
63 (implemented by skipping breakfast) reduced spontaneous light-intensity physical activity  
64 compared to when a prescribed breakfast was consumed (Betts et al. 2014; Chowdhury et al.  
65 2016), and in these studies the magnitude of this decrease in energy expenditure offset the  
66 reduction in energy intake achieved by skipping breakfast. However, it is not known whether  
67 consuming a very-low energy diet, rather than implementing a period of complete fasting, has  
68 a similar influence on habitual energy expenditure.

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

74 **2. Methods**

75 *2.1. Participants*

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

82 **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 <sup>-2</sup> )	24.59 (2.01)
Body fat (%)	16.51 (3.95)
Dietary restraint <sup>1</sup>	6 (2)
Dietary disinhibition <sup>1</sup>	9 (4)
Hunger <sup>1</sup>	7 (2)
Resting metabolic rate (kcal) <sup>2</sup>	1841 (114)

<sup>1</sup>Three factor eating questionnaire (Stunkard and Messick, 1985)

<sup>2</sup>Estimated via predictive equation (Mifflin et al. 1990)

83

84

85        2.2. *Study design*

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

93        2.3. *Preliminary trial and standardisation procedures*

94        During the preliminary trial, participants body mass (Adam CFW150; Adam Equipment  
95        Limited; Milton Keynes; UK), height (Seca; Hamberg; Germany) and skin-fold thickness  
96        (bicep, tricep, subscapular, iliac crest; Harpenden, West Sussex, UK) were measured, with BMI  
97        and body fat percentage (Durnin and Womersley, 1974) calculated. This was followed by an  
98        incremental ambulatory exercise test, with participants completing 3-minute stages, at 3.2 km/h,  
99        5.2 km/h, 5.6 km/h at 10% gradient and 9 km/h (Templeman et al. 2018; Brage et al. 2007). In  
100        the final minute of each stage, mean heart rate was recorded (Polar H10, Polar, Warwick, UK)  
101        and expired gas was collected into a Douglas bag. Expired gas was analysed for oxygen and  
102        carbon dioxide concentration (MiniMP 5200, Servomex, East Sussex, UK), volume (Dry gas  
103        meter, Cranlea, Birmingham, UK) and temperature (Digital thermometer, Fisher Scientific Ltd,  
104        Loughborough, UK), with energy expenditure calculated via indirect calorimetry (Frayn, 1983).  
105        This data was then used to individually calibrate physical activity monitors (Actiheart,  
106        CamNtech, Cambridge, UK). Participants were also fully familiarised with all *ad-libitum* buffet  
107        procedures during this preliminary trial.

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

#### 113 2.4. Protocol

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

119 After baseline measures on day 1, an Actiheart monitor was fitted and participants completed  
120 an appetite questionnaire. Participants were then informed of the diet they would receive on  
121 day 2. For the EB trial, participants were told: “*Tomorrow you will consume a diet providing*  
122 *100% of your energy (calorie) requirements. This will contain [participants standardised diet*  
123 *energy content to maintain energy balance rounded to nearest 10 kcal] and will be a similar*  
124 *amount of food to what you would normally eat*”. For the ER trial, participants were told:  
125 “*Tomorrow you will consume a diet providing 25% of your energy (calorie) requirements. This*  
126 *will contain [participants standardised diet energy content to provide 25% of energy*  
127 *requirements rounded to nearest 10 kcal] and will be about one quarter of the amount of food*  
128 *you would normally eat*”. After a 5-minute interval, participants completed another appetite  
129 questionnaire, immediately followed by an *ad-libitum* breakfast (~08:30-09:00). Participants  
130 left the laboratory after breakfast and returned to the laboratory for an *ad-libitum* lunch  
131 (~12:30-13:00). After lunch, participants were provided with a selection of snacks that could

132 be consumed *ad-libitum* 14:00-18:00 and an *ad-libitum* pasta-based evening meal to be  
133 consumed at home 19:30-20:00. *Ad-libitum* water intake was permitted throughout the day and  
134 was recorded.

135 Participants returned to the laboratory on day 2 and were provided with a standardised diet  
136 providing either 100% (EB) or 25% (ER) of EER. Breakfast was consumed in the laboratory  
137 (08:30), after which participants left with all remaining food and drink items for the day, along  
138 with instructions of when to consume each item. On day 3, participants returned to the  
139 laboratory and baseline measures were repeated, after which an *ad-libitum* breakfast meal  
140 (identical to day 1) was provided (08:00-08:30).

#### 141 2.5. Standardised diets

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



156        2.6. *Ad-libitum* food and water intake

157        Energy and macronutrient intake was assessed at a multi-item breakfast (08:30-09:00), a multi-  
158        item lunch (12:30-13:00), optional snacks (14:00-18:00) and a homogenous dinner (19:30-  
159        20:00) on day 1, as well as a multi-item breakfast on day 3 (08:30-09:00). Amounts consumed  
160        at each meal were quantified by weighing each food item before and after consumption, with  
161        energy and macronutrient intake ascertained from manufacturer values. Breakfast and lunch  
162        meals were served in the laboratory in an isolated feeding booth with no interaction between  
163        participants and investigators. Food was provided in excess of expected consumption, with  
164        more food available on request. Participants were given 30 minutes to eat each meal, and were  
165        explicitly instructed to eat until they felt “*comfortably full and satisfied*”. Items provided for  
166        each *ad-libitum* eating occasion are detailed in **Table 2**. The dinner meal was a homogenous  
167        main meal consisting of pasta, Bolognese sauce and olive oil, with chocolate-chip cookies for  
168        dessert, which participants consumed at home. The main meal was prepared the day prior to  
169        trials using identical cooking and cooling procedures, and was provided in a large plastic  
170        container. Participants were required to select a portion and warm it before eating. Participants  
171        were asked to eat this meal from the same plate or bowl during both trials, which they could  
172        refill as often as desired within 30 minutes, and they were asked to eat until they felt  
173        “*comfortably full and satisfied*”. Chocolate-chip cookies were provided in the same container  
174        and in the same quantity for both trials. All items consumed outside the laboratory were  
175        weighed before being provided and reweighed upon return to the laboratory on day 3 of the  
176        trial. Water intake was permitted *ad-libitum* and was recorded.

177

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

<b>Item</b>	<b>Energy density (kcal·100g<sup>-1</sup>)</b>	<b>Approx. amount provided (g)</b>	<b>Item</b>	<b>Energy density (kcal·100g<sup>-1</sup>)</b>	<b>Approx. amount provided (g)</b>
<i>Breakfast items (08:30-09:00)</i>					
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
<i>Lunch items (12:30-13:00)</i>					
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			
<i>Snack items (14:00-18:00)</i>					
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
<i>Dinner items (19:30-20:00)</i>					
Pasta (cooked)	176	1100	Olive oil	900	32
Bolognese sauce	45	400	Chocolate chip cookies	491	200

180        *2.7. Energy expenditure*

181 Energy expenditure was assessed in 15 second epochs from 08:30 on day 1 until 08:30 on day  
182 3 via an Actiheart monitor, which integrates heart rate and accelerometry to yield the most  
183 accurate estimation of physical activity energy expenditure of any wearable device  
184 (Chowdhury et al. 2017). Dietary induced thermogenesis was estimated from participants  
185 macronutrient intake during trials (Westterterp, 2004), then added to physical activity energy  
186 expenditure derived from the Actiheart to summate total non-resting energy expenditure. To  
187 improve the validity of the energy expenditure estimation, monitors were individually  
188 calibrated using the heart rate-energy expenditure regression equation from the sub-maximal  
189 ambulatory test conducted during the preliminary trial (Brage et al. 2007). Data was considered  
190 valid if less than 10% of the activity trace was ‘lost’ during waking hours, and <30% of the  
191 heart rate trace was ‘interpolated’ by the software (Edinburgh et al. 2019). All Actiheart data  
192 collected in the present study met this criteria, so all data were included in analysis.

193        *2.8. Subjective appetite sensations*

194 Hunger, fullness, desire to eat (DTE), prospective food consumption (PFC) and nausea were  
195 assessed via a questionnaire, immediately before and after each meal (excluding snacks). An  
196 additional questionnaire was provided before and 5 minutes after participants were informed  
197 which trial they were completing on day 1. Ratings were provided on a 100 mm visual analogue  
198 scales with anchors of “not at all/ none at all/ no desire at all” and “extremely/ a lot” placed at  
199 0 and 100 mm, respectively.

200        *2.9. Statistical analysis*

201 Data were analysed using SPSS 24.0 (IBM, Chicago, USA). All data was checked for normality  
202 using a Shapiro-Wilk test. Energy intake, macronutrient intake and PAEE data were analysed  
203 as a total for each trial and as a sub-total for each day of the study separately. Energy and

204 macronutrient intake was also analysed at each individual *ad-libitum* eating occasion. In each  
205 case, data were expressed as a single value for each trial and analysed using a paired samples  
206 *t*-test (normally distributed data) or Wilcoxon-Signed Rank test (non-normally distributed data),  
207 as appropriate. PAEE data were also sub-divided and analysed by times-of-day, i.e. early  
208 morning (06:00-08:59), morning (09:00-11:59), afternoon (12:00-16:59), evening (17:00-  
209 21:59) and overnight (22:00-05:59), and by accepted thresholds for intensity (Haskell et al.  
210 2007), i.e. sedentary (<1.5 METS), light (1.5-2.9 METS), moderate (3-5.9 METS) and  
211 vigorous (>6 METS). Similarly, data for each sub-division were expressed as a single value  
212 for each and analysed using a paired samples *t*-test or Wilcoxon Signed-Rank test, as  
213 appropriate. Repeated measures ANOVA were used to evaluate main effects of time, trial and  
214 time-by-trial interactions for variables with multiple time points (e.g. hunger, fullness, desire  
215 to eat, prospective food consumption, blood glucose and body mass). Where interaction effects  
216 were observed, Holm-Bonferroni adjusted *post-hoc* paired *t*-tests or Wilcoxon Signed-Ranks  
217 tests were conducted. For appetite-related variables, area under the curve (AUC) was calculated  
218 using the trapezoidal method, and were analysed using a *t*-test or Wilcoxon Signed-Rank test,  
219 as appropriate. Data sets were determined to be statistically significantly different when  $P<0.05$ .  
220 Data are presented as mean (SD) in text and tables and as mean (SEM) in figures.

221

222 **3. Results**

223 *3.1. Energy and macronutrient intake*

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

230 On day 2, when each participant's food intake was prescribed and provided, energy intake was  
231 2065 (118) kcal lower on ER compared to EB (**Figure 1**).

232 At breakfast on day 3, *ad-libitum* energy intake was 17% greater (176 (226) kcal) during ER  
233 compared to EB ( $P<0.05$ ). This was again driven by greater carbohydrate intake during ER  
234 ( $P<0.001$ ), with no differences in protein ( $P=0.141$ ), fat ( $P=0.179$ ) or fibre ( $P=0.885$ ) intake  
235 between trials. Water intake tended to be greater on ER compared to EB ( $P=0.067$ ; **Table 3**).

236 When comparing *ad-libitum* energy intake between the identical breakfast meals provided on  
237 day 1 and 3, there was a time ( $P<0.01$ ), a trial ( $P<0.05$ ) but no interaction effect ( $P=0.352$ )  
238 identified. Across both trials, *ad-libitum* energy intake was 16% greater during day 3 compared  
239 to day 1 (163 (227) kcal;  $P<0.001$ ). Energy intake was also 16% (135 (254) kcal) greater on  
240 day 1 and 3 combined during ER, compared to EB ( $P<0.01$ ).

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

245

246 [Figure 1 here]

247

248 **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
<b>Energy</b>	4322	4582	2755	691	1244	1420
<b>(kcal)</b>	(1065)	(1193)†	(159)	(42)†	(567)	(596)†
<b>Protein (g)</b>	140	148	126	80	48	53
	(35)	(41)	(8)	(5)†	(23)	(21)
<b>CHO (g)</b>	588	626	367	69	187	215
	(143)	(149)†	(23)	(4)†	(88)	(95)†
<b>Fat (g)</b>	148	156	84	10	31	36
	(49)	(53)	(6)	(1)†	(17)	(19)
<b>Fibre (g)</b>	41	42	15	5	12	13
	(12)	(10)	(2)	(0)†	(6)	(4)
<b>Water (g)</b>	4568	5217	3488	3215	974	1064
	(839)	(1241)†	(304)	(295)†	(276)	(336)

249 *Values are means (SD). EB: energy balance trial; ER: energy restriction trial. CHO;*

250 *carbohydrate. † indicates significantly different from EB (P<0.05).*

251

### 252 3.2. Energy expenditure

253 PAEE was 11% lower on day 1 (1221 (474) vs. 1064 (436) kcal;  $P<0.05$ ) and 18% lower on

254 day 2 (1183 (409) vs. 944 (370) kcal;  $P<0.05$ ) during ER compared to EB (**Figure 2**). Over the

255 study period, PAEE was 16% lower during ER compared to EB (2403 (700) vs. 2008 (692)  
256 kcal;  $P<0.01$ ).

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

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

270 Over the study period, PAEE was 395 (452) kcal lower during ER compared to EB ( $P<0.01$ ),  
271 which was sufficient to replace 19% of the energy deficit created by the energy restriction  
272 intervention on day 2. Accounting for differences in dietary induced thermogenesis between  
273 trials, energy expenditure was 521 (469) kcal lower during ER ( $P<0.001$ ), compensating for  
274 25% of dietary induced energy deficit achieved on day 2.

275

276 **[Figure 2 here]**

277

278 3.3. Subjective appetite sensations

279 There were trial ( $P<0.001$ ), time ( $P<0.001$ ) and interaction ( $P<0.001$ ) effects for hunger,  
280 fullness, DTE and PFC. There were no trial ( $P=0.334$ ), time ( $P=0.418$ ) or interaction ( $P=0.393$ )  
281 effects for nausea. On day 1, there was a tendency for DTE to be lower before lunch ( $P=0.059$ )  
282 and fullness was greater after dinner and before bed ( $P<0.05$ ) during ER compared to EB.  
283 Informing participants that they were completing the ER trial did not immediately influence  
284 fullness, DTE or PFC ( $P>0.403$ ), but tended to increase hunger ( $P=0.088$ ), and there was no  
285 immediate effect on any marker of appetite when they were told they were completing the EB  
286 trial ( $P>0.276$ ). AUC over the entire day was greater for DTE during EB compared to ER  
287 ( $P<0.05$ ), but there was no difference in AUC for hunger ( $P=0.370$ ), fullness ( $P=0.205$ ), PFC  
288 ( $P=0.594$ ) or nausea ( $P=0.791$ ; **Figure 3**).

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

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

301



302 [Figure 3 here]

303

304 3.4. Body mass and blood glucose concentration

305 There were time ( $P<0.001$ ) and interaction ( $P<0.001$ ) effects, but no effect of trial ( $P=0.713$ )  
306 for body mass. Body mass on day 3 was 0.7 (0.7) kg lower on ER compared to EB ( $P<0.01$ ;  
307 **Table 4**). Between day 2 and day 3, body mass decreased during ER ( $P<0.001$ ) and tended to  
308 decrease during EB ( $P=0.094$ ). The amount of body mass lost between day 2 and 3 was  
309 considerably greater during ER compared to EB (1.4 (0.7) kg vs. 0.7 (0.7) kg;  $P<0.0001$ ). Body  
310 mass also increased by 0.5 (0.7) kg between day 1 and 2 during ER ( $P<0.05$ ).

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

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

	<u>Energy Balance (EB)</u>			<u>Energy Restriction (ER)</u>		
	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 <sup>-1</sup> )	4.38 (0.41)	4.51 (0.29)	4.45 (0.27)	4.50 (0.37)	4.43 (0.47)	4.28 (0.30)

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

318

319 **4. Discussion**

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

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

337 The results of the present study indicate that knowledge of a future period of severe energy  
338 restriction, as would be the case in a real-world setting, results in an anticipatory increase in  
339 energy intake. In the present study, participants increased their energy intake by ~260 kcal on  
340 day 1, essentially compensating for ~12% of the energy deficit, before even undertaking the 24  
341 h period of severe energy restriction on day 2. This data has implications for intermittent fasting  
342 diets that involve alternating between periods of severely restricted food intake and periods of

343 *ad-libitum* food intake. A popular variation of intermittent fasting is the 5:2 diet, which  
344 typically involves splitting the seven-day week into two days of severely restricted food intake  
345 (~500 kcal) and five days of unrestricted eating (Harvie and Howell, 2017). The day-to-day  
346 flexibility of this method is thought to be one of the key reasons for its popularity (Harvie and  
347 Howell, 2017), but consequently, this presents opportunities for individuals to increase energy  
348 intake above their adequate energy requirements outside of the defined period of restriction.  
349 Previous studies have demonstrated that compensatory eating occurs *after* a period of severe  
350 energy restriction, but the present study provides novel findings that compensatory eating also  
351 occurs *before* a period of severe energy restriction has commenced. This is likely to reduce the  
352 magnitude of the energy deficit achieved.

353 Appetite is thought to be governed by homeostatic, environmental and cognitive factors that  
354 culminate in the initiation and termination of an eating episode. It is therefore interesting to  
355 note that, despite consuming more food, participants did not report any orexigenic differences  
356 in appetite sensations during day 1 of the ER trial, in the present study. Indeed, the only  
357 observed difference in appetite on day 1 was a reduced desire to eat during the ER trial, which  
358 likely reflects the fact that participants consumed more food during the ER trial. This difference  
359 in desire to eat may have also been magnified by the fact that appetite was only assessed before  
360 and after each meal. The energy intake results align closely with an alternative theory on eating  
361 behaviour, termed 'expected satiety', in that meal size is determined in advance of an eating  
362 occasion (Brunstrom, 2011). In the context of the present study, participant's expectations on  
363 how satiated (or hungry) they would feel on day 2 may have influenced their eating behaviour  
364 on day 1. Recently, Potter et al. (2019) found that individuals who were not successful with  
365 intermittent fasting reported that they were more likely to eat in anticipation of a future need,  
366 compared to individuals currently undertaking intermittent fasting. The participants of the  
367 present study were healthy males not currently undertaking any weight management

368 programme and were not accustomed to intermittent fasting diets. It would be interesting to  
369 determine whether eating behaviour differed after repeated exposure to periods of severe  
370 energy restriction, particularly as expected satiety is modulated by previous experience, which  
371 may influence portion size selection (Brunstrom et al. 2008).

372 The present study also observed a decrease in PAEE in the 24 h before, and during, the period  
373 of severe energy restriction. In essence, this reduction in PAEE served to reduce the energy  
374 deficit achieved by the dietary energy restriction study intervention on day 2 by ~400 kcal  
375 (16%), compared to the energy balanced control trial. The attenuation in PAEE during the  
376 period of severe energy restriction may be the result of perceived lethargy or reduced substrate  
377 availability, resulting in either the conscious or subconscious reduction of non-essential  
378 physical activity (Betts et al. 2016). Previous studies have reported a similar reduction in PAEE  
379 in response to extended morning fasting (Betts et al. 2014; Chowdhury et al. 2016). In one  
380 study, average daily PAEE was ~440 kcal greater in lean individuals who consumed a 700 kcal  
381 breakfast for six weeks, compared to individuals who skipped breakfast and fasted until midday  
382 (Betts et al. 2014). Of note, a significant proportion of this difference (~180 kcal) occurred  
383 before midday, coinciding with the time when no energy was consumed in the breakfast  
384 skipping group (Betts et al. 2014). The current study provides an important addition to the  
385 literature, as the reduction in PAEE detected using combined heart-rate accelerometers (i.e.  
386 Actiheart monitors) on day 1 cannot be attributed to a fasting-related reduction in heart rate  
387 (Matsumoto et al. 2001), as could be suggested with previous studies. Therefore, in conjunction  
388 with previous studies, these findings provide strong evidence that complete or severe energy  
389 restriction leads to a conscious or subconscious concurrent reduction in PAEE. In the context  
390 of obesity, it should be noted that 10% weight gain achieved by over-nutrition was associated  
391 with an increase in energy expenditure, which could not be fully explained by an increase in

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

394 To our knowledge, this is the first study to observe a reduction in PAEE in anticipation of a  
395 period of severe energy restriction in humans. These findings indicate that PAEE is not solely  
396 affected by concurrent nutrient availability, but also regulated in response to a threat to energy  
397 homeostasis. This may stem from an evolutionary trait in humans to conserve energy in  
398 preparation for periods of reduced food availability (Leiberman, 2006). In the wild, when food  
399 availability is intermittent, some animals will moderate their non-essential thermogenesis,  
400 enabling their endogenous energy reserves to sustain them for the longest time possible (Halsey,  
401 2016). In addition, animals that gorge on food when there is an abundance, will increase their  
402 energy expenditure as a means of maintaining a stable body weight (Halsey, 2018). These  
403 examples highlight that sustaining a healthy body weight is of critical importance for wild  
404 animals, likely because a fluctuation may make them vulnerable to predators or reduce  
405 reproductive proficiency (Halsey, 2016). These are generally not concerns shared by humans  
406 in the modern world, but it is well-established that the appetite regulatory system is sensitive  
407 to an energy deficit, but less so to an energy surplus (Rogers and Brunstrom, 2016), suggesting  
408 a disproportionate response in humans which favours weight gain. An interesting extension to  
409 this work would be to consider participants subjective psychological responses to energy  
410 restriction, which would help to determine the extent to which PAEE is consciously altered.

411 Linked to this, one study reported that misleading participants to believe that they would not  
412 be eating breakfast resulted in an increase in fasting concentrations of the orexigenic hormone  
413 ghrelin, which remained elevated post-prandially even after participants had consumed  
414 breakfast (Ott et al. 2012). Ghrelin has also been suggested to be involved in the regulation of  
415 physical activity via the hypothalamic neuropeptide AgRP (Pfluger et al. 2011), suggesting  
416 there is interplay between mechanisms affecting components of energy balance. Whilst beyond

417 the scope of this study, alterations in appetite hormone concentrations occurring after  
418 participants were informed of their day-2 diet may lead to reduced physical activity via this  
419 pathway, although future studies would be required to elucidate a mechanistic link between  
420 anticipatory appetite and physical activity.

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

433 The current study found an increase in food intake with a concurrent reduction in physical  
434 activity in *anticipation* of severe energy restriction (by ~390 kcal), a reduction in physical  
435 activity *during* the period of energy restriction (by ~435 kcal) and an increase in energy intake  
436 at the first meal following the period of energy restriction (by ~175 kcal). In total, these  
437 compensatory changes accounted for 1108 (415) kcal of the 2065 (118) kcal reduction in  
438 energy intake achieved by the severe energy restriction intervention on day 2. Considering also  
439 that other similarly designed studies have observed further compensations in energy intake, in  
440 the 48h following a period of severe energy restriction (Clayton et al. 2016; Clayton et al. 2016),  
441 it is clear that the magnitude of the energy deficit achieved by severe energy restriction would

442 be reduced considerably by compensatory behavioural alterations. Physical activity and ‘binge’  
443 eating are also associated with chronic disease (Roberts and Bernard, 2005; Parry et al. 2017),  
444 therefore, even if the energy deficit conserved after compensation is still sufficient to prevent  
445 weight gain, future studies will need to determine the impact of these behaviours on long-term  
446 metabolic health.

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

461 In conclusion, the current study has shown that compensatory changes in physical activity  
462 energy expenditure and energy intake may occur before, during and after a period of severe  
463 energy restriction, and these changes serve to reduce the magnitude of the energy deficit that  
464 is achieved by severe energy restriction. These results suggest that flexible intermittent diets  
465 that incorporate severe energy restriction interspersed with periods of unrestricted intake may  
466 induce a smaller energy deficit than anticipated, which may have implications for long-term

467 weight management. Future studies should aim to develop strategies to mitigate against energy  
468 compensation during intermittent dieting, with the current study indicating these strategies  
469 should be implemented before and after periods of energy restriction, and target both sides of  
470 the energy balance paradigm.

471 **Declaration of competing interests**

472 None

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476 **Author contributions**

477 DJC, LJJ and RJ designed the study. RJ and DJC performed data collection and analysis. DJC  
478 and RJ wrote the manuscript with assistance from LJJ. All authors approved the final version  
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612

613 **Figure Captions**

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

621

622 **Figure 2:** Physical activity energy expenditure during day 1 (left) and day 2 (right) during the  
623 energy balance (EB) and energy restriction (ER) trials. Total energy expenditure is sub-divided  
624 by activity intensity (a) – sedentary (black bar), light (grey bar), moderate (white bar) and  
625 vigorous (crosshatch bar) and by time of day (b) – early morning (06:00-08:59), morning  
626 (09:00-11:59), afternoon (12:00-16:59), evening (17:00-21:59) and overnight (22:00-05:59). †  
627 indicates a significant difference in total physical activity energy expenditure between ER and  
628 EB during the corresponding day ( $P<0.05$ ). P-values between the bars represent the comparison  
629 between trials for energy expenditure at the each intensity/time of day during the corresponding  
630 day. Values are mean (SEM).

631

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