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1 *Intermittent energy restriction is comparable to continuous energy restriction for cardiometabolic health*
2 *in adults with central obesity: a randomized controlled trial; The Met-IER Study.*

3

4 **28th June 2019**

5

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14 ABSTRACT

15 **Background & aims:** Short bouts of severe energy restriction may have additional, beneficial
16 cardiometabolic effects beyond that of weight loss. **We aimed** to assess the short-term effects of intermittent
17 fasting on insulin sensitivity and related cardiometabolic mechanisms.

18 **Methods:** This parallel arm, randomized controlled trial compared the short-term effects of intermittent and
19 continuous energy restriction (IER and CER) diets on markers of cardiometabolic health in individuals with
20 central obesity, aiming for equivalent weight loss on both diets. Outcomes were assessed in non-smoking
21 men and women (35–75 y), following 4-wk IER (48 h 600 kcal/d followed by 5-day healthy eating advice)
22 or CER diets (-500 kcal/d healthy eating advice). The primary outcome was the revised quantitative insulin
23 sensitivity check index (R-QUICKI), an indirect estimate of insulin sensitivity. Secondary outcomes
24 included ambulatory blood pressure (ABP), indicators of sympathetic activity (heart rate variability (HRV)
25 and normetanephrine), and markers of glucose homeostasis/insulin resistance, adiposity, lipids and
26 inflammation.

27 **Results:** Forty-three participants completed the study. Reductions in body weight were equivalent in both
28 groups: mean loss (%) -2.6; 95% CI -3.3, -1.9 and -2.9; -3.6, -2.1 for CER and IER, respectively, $P = 0.464$).
29 R-QUICKI increased following IER and CER, with no between-diet differences (overall mean increase (%)
30 6.6; 3.6, 9.6). Fasting plasma glucose concentrations decreased after CER but not after IER (mean difference
31 CER - IER - 4.8% (0.7, 8.9), $P < 0.05$) and fasting plasma non-esterified fatty acid concentrations were lower
32 after IER compared to CER (mean difference CER - IER 0.15 mmol/L (0.06, 0.24), $P < 0.005$). There were no
33 differences in lipids, adipokine/inflammatory markers, ABP or HRV between diets.

34 **Conclusions:** Short-term CER or IER diets are comparable in their effects on most markers of
35 cardiometabolic risk, although adaptive changes in glucose and fatty acid metabolism occur. This study is
36 registered at clinicaltrials.gov as NCT02679989.

37

38 **Key words:** Intermittent energy restriction; randomized controlled trial; central obesity; insulin sensitivity;
39 heart rate variability; cardiometabolic health.

40

41 *Abbreviations*

42 Ambulatory blood pressure, ABP; Beta-hydroxybutyrate, β -OHB; body fat, BF; body mass index, BMI;
43 continuous energy restriction, CER; Dutch Eating Behaviour Questionnaire, DEBQ; diastolic blood pressure,
44 DBP; high density lipoprotein, HDL; homeostasis model assessment of insulin resistance, HOMA-IR; heart
45 rate, HR; heart rate variability, HRV; high frequency power, HF; intermittent energy restriction, IER; low
46 density lipoprotein, LDL; mean arterial pressure, MAP; non-esterified fatty acids, NEFA; Revised
47 Quantitative Insulin Sensitivity Check Index, RQUICKI; the square root of the mean of the sum of squares
48 of differences between adjacent normal-to-normal intervals, RMSSD; resting metabolic rate, RMR; severe
49 energy restriction, SER; standard deviation of all normal-to-normal intervals, SDNN; systolic blood pressure,
50 SBP; triacylglycerol, TAG; very low calorie diet, VLCD

51 INTRODUCTION

52 Obesity and overweight are associated with increased risk of chronic diseases, including type 2
 53 diabetes (T2D) and cardiovascular diseases (CVD) [1]. Central obesity is the central feature of metabolic
 54 syndrome and confers a greater risk of cardiometabolic diseases due to the primary role of intra-abdominal
 55 fat in inflammation and insulin resistance [2,3]. Excess visceral adipose tissue results in a greater amount of
 56 fatty acids being delivered to the liver, promoting hepatic insulin resistance, inflammation, and
 57 hypertriglyceridaemia [4]. Visceral fat is rapidly lost in the early stages of weight loss interventions [5]; very
 58 low calorie diets (VLCD) and moderate weight loss interventions often result in rapid reductions in plasma
 59 triacylglycerol (TAG) [6], blood pressure [7] and fasting insulin concentrations/insulin resistance [8,9];
 60 within a few weeks. Additionally, diet-induced weight loss can significantly increase heart rate variability
 61 (HRV), indicating improved autonomic function [10]. Abdominal visceral fat has been strongly associated
 62 with sympathetic nervous system (SNS) activation [11], a key factor in the pathogenesis of obesity-related
 63 insulin resistance and hypertension [12,13]. Adults with obesity have raised urinary norepinephrine levels
 64 and lower HRV, indicating greater sympathetic outflow, compared to healthy individuals [14,15].
 65 Furthermore, adults with central obesity presented a higher degree of sympathetic activation when compared
 66 to individuals with subcutaneous obesity [16]. The elevation in sympathetic activity associated with obesity
 67 may be partly responsible for impairments in insulin-mediated glucose uptake, leading to compensatory
 68 increases in insulin secretion that in turn further exacerbates sympathetic over-activity [17].

69 Intermittent energy restriction (IER) is an increasingly popular alternative to the orthodox
 70 continuous energy restriction (CER) approach to weight loss [18,19]. IER diets comprise a predefined period
 71 (24-48 h) of severe energy restriction (SER), alternated with a period of habitual energy intake. The most
 72 common energy restriction patterns that come under the IER category include alternate day fasting [20] and
 73 the 5:2 approach: 5 days of unrestricted eating combined with 2 days of SER each week [21]. A number of
 74 systematic reviews have recently reported that there is no evidence that IER interventions of 5 weeks to 12
 75 months are more effective than CER interventions in treating overweight/obesity [21–24], and a few
 76 randomized controlled trials have also reported similar improvements in blood pressure, fasting glucose and
 77 lipids, and inflammatory markers [25–28]. However, it has also been reported that a 5:2 diet (with
 78 consecutive SER days) causes greater reductions in fasting insulin and insulin resistance compared to CER in
 79 women who were overweight and obese after 3 and 6 months interventions [29,30].

Twenty-four hours of SER typically decreases plasma fasting glucose concentrations and depletes glycogen stores, causing a metabolic shift to fatty acid oxidation, fuelled by free fatty acids mobilised from fat stores (including visceral adipose tissue), and synthesis of ketone bodies as energy sources. Two consecutive SER days will induce marked fluctuations in ketone body production, and it is hypothesised in this paper that this provides acute cardiometabolic benefits. β -hydroxybutyrate (β -OHB), a ketone body, is a ligand for free fatty acid receptor 3 (FFAR3), a G-protein coupled receptor that regulates sympathetic tone [31], presenting a plausible mechanism for the hypothesis that IER is more effective than CER in improving insulin sensitivity, lowering blood pressure and optimising autonomic function via inhibition of sympathetic activity.

This study aimed to compare short-term changes in markers of cardiometabolic health following isoenergetic IER and CER diets in adults with central obesity. To distinguish energy restriction pattern effects, equivalent weight loss following both arms was an objective of the study protocol. The IER regime comprised 2 consecutive days of SER, in order to induce higher circulating β -OHB concentrations than CER. The primary hypothesis was that IER would increase insulin sensitivity to a greater extent than CER, independently of weight loss. Secondary outcome variables included markers of sympathetic nervous system activity, glucose and lipid homeostasis, inflammation and body composition.

96

97 MATERIAL AND METHODS

98 Subjects

Participants were recruited through London-wide newspaper advertisements and electronic internal circulars at King's College London. The main inclusion criteria were non-smoking men and women aged 35-75 years with a waist circumference exceeding the cut-off determined by the World Health Organisation to confer a high risk of cardiometabolic disease [32]: >102 cm and >88 cm for men and women respectively (>90 cm and >80 cm, for men and women respectively, with South Asian or East Asian ethnic background [33]). There were no inclusion/exclusion criteria based on BMI since this index does not provide information on body fat distribution. The exclusion criteria included kidney or cardiovascular disease, cancer, diabetes, chronic liver disease; previous bariatric surgery or other major surgery (e.g. organ transplantation); significant psychiatric disorder or uncontrolled depression; eating disorders; participation in a weight management drug trial in the previous 3 months; uncontrolled epilepsy; taking medication likely to affect

109 metabolic rate and/or weight (e.g. beta blockers, corticosteroids, diuretics); lactose intolerant; alcohol or
 110 substance abuse. Women who were currently pregnant, lactating or planning pregnancy were also excluded.
 111 This study was conducted according to guidelines laid down in the Declaration of Helsinki and all
 112 procedures involving human subjects were approved by the Research Ethics Committee (REC) of King's
 113 College London (HR-15/16-2179). Participants gave written informed consent before participation and
 114 received a small remuneration for taking part. This study was registered at clinicaltrials.gov as
 115 NCT02679989.

116

117 **Study protocol**

118 The Met-IER study (The Impact Of An Intermittent Energy Restricted Diet On Insulin Sensitivity In
 119 Men and Women With Central Obesity), was a 4-week parallel arm randomized controlled trial designed to
 120 compare the relative cardiometabolic effects of short-term IER and CER in men and women with central
 121 obesity. The study was conducted at the metabolic research unit (MRU) at King's College London, United
 122 Kingdom, between February and July 2016. Potential participants attended a screening visit upon which
 123 anthropometric and resting blood pressure measurements were taken, alongside a fasting blood sample to
 124 assess lipid profile, glucose, liver function and haematology to confirm eligibility to take part in the study.
 125 Before randomisation to treatment, eligible participants were asked to complete a 7-day food record and a
 126 validated eating habits questionnaire (Dutch Eating Behaviour Questionnaire, DEBQ), which evaluates
 127 dietary restraint and emotional/external eating [34]. The DEBQ was used to assess disordered eating
 128 behaviour and to check that the groups were balanced for eating styles. Treatment was randomly allocated by
 129 the lead researcher using a computer online MinimPy 0.3 (Copyright (c) 2011 Mahmoud Saghaei,
 130 <http://minimpy.sourceforge.net>) by minimization for sex, BMI, ethnicity and waist circumference.
 131 Participants attended the MRU in the fasted state for baseline measurements of body composition, resting
 132 blood pressure, resting and post-mental stress HRV, resting metabolic rate (RMR), to provide fasting blood
 133 samples, and to be fitted with 24 h ambulatory blood pressure (ABP) and HRV monitors; participants also
 134 completed the COPE inventory [35], designed to assess coping strategies to respond to stress, and performed
 135 a Mnemonic Similarity Task (MST) [36]. The MST was carried out as a collaboration with the Institute of
 136 Psychiatry (IoP) at King's College London (Denmark Hill campus) as an exploratory investigation into
 137 effects on memory performance, hence the results will be reported elsewhere. All the procedures were

repeated in duplicate at endpoint after 4-5 weeks of dietary intervention, depending on participants availability. Duplicate end-points included post-2-d SER, and also post-“normal eating” (for a minimum of 2 d) whilst maintaining moderate energy restriction (non-SER), in order to investigate acute effects of SER within the IER group. The order of the endpoint visits (after 2-d SER, and after non-SER) was arranged to suit each individual for their convenience. The CER group also had duplicate end-point measurements in order to match with the IER group. The timeline for the study is shown in **Supplementary Figure 1**.

Both dietary intervention arms were designed to reduce weekly energy intake by 3500 kcal (14.64 MJ) relative to estimated total energy expenditures, in order to elicit body weight loss of at least 2 kg following the 4-week intervention. Total energy expenditure (TEE) was calculated from resting metabolic rates measured by indirect calorimetry using the FitMate™ (COSMED, Rome, Italy), a previously validated [37,38] metabolic analyser that measures O₂ consumption under a hood to estimate energy expenditure. Measured RMRs were entered into an adapted version of a previously published spreadsheet with sex-specific algorithms [39] along with variables including age, weight and time spent doing different levels of physical activities estimated using an adaptation of the international physical activity questionnaire – short form (IPAQ-SF) [40]. RMR was also measured at endpoint to assess whether there was any difference between diets in the degree of compensatory reduction in metabolic rate that commonly occurs with weight loss [28]. Participants allocated to CER were given personalised dietary advice to consume a nutritionally-balanced Mediterranean-style diet incorporating a daily 500 kcal (2.09 MJ) deficit relative to estimated TEE. Individuals in the energy-matched IER group were asked to follow a VLCD diet on 2 consecutive days (SER days) each week based on commercially available meal replacement food packs (600 kcal, or 2510 kJ per day providing an average of 38%, 36% and 26% of total energy as carbohydrate, protein and fat, respectively, and 100% recommended daily allowances for vitamins and minerals) supplied by LighterLife UK Ltd (Harlow, UK). Participants were able to choose their preferred food packs from a range of options, including milkshakes, soups, porridge, sweet bars and savoury dishes. On the remaining 5 days of the week individuals were asked to follow a nutritionally-balanced, energy-controlled Mediterranean-style diet, with a target of the same weekly energy deficit as the CER group. All participants received personalized advice for dietary changes based on information provided in their baseline 7-day food records and information provided verbally. For example, they were advised to choose unsaturated instead of saturated fats (e.g. olive oil, rapeseed oil and sunflower oil) and limit it to 1 tsp per meal, and mid-morning snacks could be either a piece

167 of fruit, 1 rice cake with 1 tsp peanut butter, or 1 tbsp nuts. Moreover, one physical activity goal was set for
 168 all participants to keep throughout the trial depending on the baseline physical activity frequency and
 169 intensity reported. To support compliance, all participants received motivational phone calls and were
 170 encouraged to obtain peer/family support to maintain diets. One-hour group support sessions were provided,
 171 consisting of educational talks on portion sizes and problem resolution combined with interactive tasks to
 172 reinforce messages on portion sizes. The power of food scale, which measures appetite drive to consume
 173 highly palatable food [41], was used in the group session in order to aid debate on potential barriers to
 174 compliance with the dietary advice and strategies to overcome the identified barriers. Mean daily intakes
 175 were analysed using Nutritics (Nutritics Professional Diet Analysis version 3.06, Nutritics Ltd., Ireland),
 176 which incorporates the 6th edition of McCance and Widdowson's Composition of Foods database.

177

178 **Body weight and composition**

179 Weight and body composition were estimated using bioelectrical impedance scales (Tanita BC-418
 180 MA; Tanita, Arlington Heights, IL, USA). Waist circumference (WC) and hip circumference (HC) were
 181 measured around the umbilicus and the widest point over the buttocks, respectively, using a non-stretch
 182 measuring tape.

183

184 **Clinical and laboratory parameters**

185 Fasting blood samples were immediately centrifuged and plasma/serum was frozen at -40°C or -
 186 80°C until analysis. All blood analyses were determined by a clinical pathology accredited laboratory
 187 (ViaPath, Kings College Hospital). Plasma glucose and serum lipids (total cholesterol, HDL-cholesterol,
 188 TAG, non-esterified fatty acids (NEFA)) were analysed following enzymatic methods using reagents
 189 supplied by Bayer Diagnostics Europe Ltd (Bayer House) using an ADVIA 2400 analyser (Siemens
 190 Healthcare Diagnostics). LDL-cholesterol was calculated using the Friedewald formula. ELISAs were used
 191 to analyze serum insulin (Siemens Healthcare Diagnostics Ltd, Frimley, Surrey, UK), adiponectin and leptin
 192 (Quantikine ELISA kits, R&D Systems, Abingdon, UK). Serum β -OHB was analysed using an enzymatic
 193 assay supplied by Randox Laboratories Ltd (County Antrim, UK) for the ADVIA 2400 analyser.
 194 Inflammatory markers tumour necrosis factor (TNF)- α , interleukin (IL)-6, IL-8, VEGF, IL-1b, IL-1RA, and
 195 MCP-1 were analysed in blood plasma using a high-sensitivity cytokine chip array assay (Human cytokine

196 HS X biochip; Randox Laboratories Limited, County Antrim). Plasma free normetanephrine (NMN), the
 197 stable O-methylated metabolite of norepinephrine, was measured as an additional marker of sympathetic
 198 activity by liquid chromatography-tandem mass spectrometry using TurboFlow™, an online sample
 199 preparation system. Insulin sensitivity was assessed by the revised quantitative insulin sensitivity check
 200 index (RQUICKI: $1/(\log \text{glucose (mg/dL)} + \log \text{Insulin } (\mu\text{U/mL}) + \log \text{FFA (mmol/L)})$)[42], and insulin
 201 resistance by the homeostatic model assessment (HOMA-IR: $\text{Glucose mM} \times \text{Insulin mU/L} / 22.5$).

202 To avoid the difficulties associated with multiple testing, a summary score for low-grade
 203 inflammation was calculated by averaging z-scores of log transformed inflammatory markers as follows, an
 204 approach adapted from previously reported studies [43–45]:

205
 206
$$\text{Summary inflammatory score} = [\text{z score}_{(\text{LN TNF-}\alpha)} + \text{z score}_{(\text{LN IL-6})} + \text{z score}_{(\text{LN IL-8})} + \text{z score}_{(\text{LN VEGF})} +$$

 207
$$\text{z score}_{(\text{IL-1B:IL-1FA ratio})} + \text{z score}_{(\text{LN MCP})}$$

208

209 **Blood pressure and heart rate variability measurements**

210 Supine resting and ambulatory (24 h, day-time and night-time) systolic and diastolic blood pressure
 211 (SBP and DBP) were measured using an A&D TM-2430 ABP monitoring device (A&D Inc., Tokyo, Japan)
 212 with appropriate cuff sizes. Resting SBP and DBP were recorded in triplicate after 15 min supine rest, where
 213 the first measurement was disregarded and the mean of the second and third were used for analysis. The
 214 ambulatory readings were obtained every 30 min during day-time and every 60 min at night, over a 24-h
 215 period. All participants were asked to maintain their usual routine, to remain still when the cuff inflated
 216 during the daytime, and to record their physical activity and sleeping time in an activity diary. Upon return of
 217 ABP monitors, data were analysed with A&D Professional Analysis software, and any errors or non-
 218 physiological anomalies were excluded, before calculation of mean 24 h, day-time and night-time SBP, DBP
 219 and mean arterial pressure (MAP). A small, light-weight, chest-worn wireless 2-lead ambulatory heart
 220 rate/ECG monitor (eMotion Faros 180°, Mega Electronics Ltd., Kuopio, Finland) was fitted to measure 30-
 221 min resting, 10-min mental stressor test (the Stroop colour word test [46], in order to monitor HRV during
 222 mild mental stress as a measure of SNS activation) and 24 h ambulatory HRV. HRV data was analysed using
 223 Cardioscope™ Analytics software (HASIBA Medical GmbH, Graz, Austria). The eMotion Faros 180° also
 224 has an in-built 3-axis accelerometer that records acceleration (cpm) as a measure of physical activity.

225 Ambulatory HRV and interbeat interval (IBI) data was reported as 24 h, day-time and night-time using
 226 standardised periods of 8 h (day) and 5 h (night) to remove the influence of variability in recording duration
 227 on HRV parameters. Time-domain HRV parameters included the mean of the standard deviations of the
 228 normal-to-normal (NN) intervals (SDNN) and root mean square of successive differences of NN intervals
 229 (RMSSD). Frequency-domain HRV parameters included high-frequency (HF) power [47].

230

231 **Statistical analysis**

232 The primary endpoint of the study was R-QUICKI, a simple fasting index of insulin sensitivity that
 233 has previously been demonstrated to correlate highly with results from the euglycaemic hyperinsulinaemic
 234 clamp in lean and impaired glucose tolerance subjects [48]. Expected changes in R-QUICKI were therefore
 235 used to calculate sample size based on previously published authors' data [49]. Using a two-sided 5% level
 236 of significance and 80% power, it was determined that 17 participants per arm were required to detect a
 237 mean difference in R-QUICKI of 0.06. The goal was to recruit 23 subjects per arm in total to allow for 25%
 238 drop-out rates. Statistical analyses were performed using IBM SPSS Statistics 22.0 (Statistical Product and
 239 Service Solutions; IBM Corp.). Variables were tested for normality, and log transformed where necessary.
 240 Baseline analysis to test for differences between groups was performed using independent *t*-test, or Chi
 241 square where appropriate. For primary analysis, between-diet group differences were tested on endpoint
 242 values using ANCOVA, adjusted for baseline values, and, for non-anthropometric outcomes, % weight loss,
 243 on an intention-to-treat basis. Importantly, the endpoint (END) between-group comparisons were following
 244 at least 2 days moderate energy restriction, not SER, in order to assess the chronic effects of IER relative to
 245 CER, rather than the more transient metabolic changes than can occur after 48 h of a very low energy diet.
 246 Thus, END was defined as the average of the measurements taken at the two follow-up visits for the CER
 247 group and the measurements taken at the follow-up visit after non-SER (at least two consecutive days of not
 248 fasting) for the IER group. The 2-d SER endpoint was defined as the measurements taken at the follow-up
 249 visit after two consecutive days of 600 kcal/d energy intake. A between-diet difference was noted as
 250 statistically different when $P < 0.05$. The END results were expressed as estimated marginal means (95% CI)
 251 adjusted for % weight loss and baseline values, and in the case of 24 h and day-time HRV data, physical
 252 activity. Data that could not be normalised by LN transformation were analysed using Mann-Whitney *U* test
 253 and significance values are presented unadjusted, with results shown as medians (lower and upper limits of

254 the interquartile range). To explore any acute effects of the IER 2-d SER endpoint compared with non-SER
255 endpoint, paired sample T-tests were performed (two-tailed). To assess compliance to the dietary
256 intervention, changes in weight were assessed using paired t-test at baseline and END. To assess compliance
257 with 2-d SER, changes in serum β -OHB concentrations in the IER group were assessed using paired t-test at
258 END and after 2-d SER.

259

260 **RESULTS**

261 Forty-five volunteers were enrolled and randomized to a treatment group (**Figure 1** shows the flow
262 of participants through the study). Two participants from the IER group withdrew from the study after the
263 baseline visit due to change in availability; the remaining 43 participants completed the study.

264

265 **Figure 1.** CONSORT diagram. IER, intermittent energy restriction; CER, continuous energy restriction.

*Participants received £75 for taking part in the study.

266

267

268

269

General characteristics at screening of the completers are reported in **Table 1**. Subjects were predominantly pre-hypertensive, with an average BMI of 31 kg/m², and on average the population had raised cholesterol concentrations and normal fasting glucose concentrations. There were no significant differences

in baseline mean age, waist circumference or BMI, or distributions of ethnicity and sex, between groups. There were also no significant differences in DEBQ scores, seated resting DBP, SBP and HR between groups or biochemistry measurements (plasma glucose, serum TAG, serum total-cholesterol, LDL-cholesterol and HDL-cholesterol).

Table 1 – Subject characteristics at screening visit

	CER (N=22)	IER (N=21)	P value
Sex (m/f)	6/16	6/15	1.000
Ethnicity (White/Black/South Asian/Other)	15/3/3/1	14/1/3/3	0.570
Age (years)	56 ± 8	50 ± 12	0.097
Waist circumference (cm)			
Male	120 (110, 131)	113 (106, 120)	0.158
Female	108 (100, 116)	105 (99, 110)	0.532
BMI (kg/m²)	31.1 ± 5.7	31.8 ± 4.5	0.638
% body fat			
Male	31.2 ± 4.2	33.6 ± 6.7	0.476
Female	40.6 ± 5.7	43.1 ± 4.1	0.173
DEBQ			
Emotional	2.80 ± 1.12	2.46 ± 0.90	0.288
External	3.12 ± 0.72	3.12 ± 0.56	0.996
Restrained	2.94 ± 0.66	2.83 ± 0.72	0.586
SBP (mmHg)	132 ± 16	127 ± 14	0.325
DBP (mmHg)	88 ± 12	86 ± 8	0.491
HR (bpm)	64.1 ± 7.1	67.0 ± 7.5	0.201
Plasma glucose (mmol/L)	5.4 ± 0.5	5.3 ± 0.4	0.431
Serum TAG (mmol/L)	1.08 (0.88, 1.33)	1.17 (0.96, 1.43)	0.560
Serum total cholesterol (mmol/L)	5.6 ± 0.9	5.4 ± 1.2	0.544
Serum LDL-cholesterol (mmol/L)	3.4 ± 0.7	3.3 ± 1.0	0.719
Serum HDL-cholesterol (mmol/L)			
Male	1.31 ± 0.24	1.35 ± 0.33	0.768
Female	1.80 ± 0.38	1.61 ± 0.42	0.190

BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein; HR, heart rate; LDL, low density lipoprotein; SBP, systolic blood pressure; TAG, triacylglycerol. DEBQ scores range from 1 to 5 where the higher the score the stronger the behaviour. Results expressed as number, mean ± SD or geometric mean (95% CI)

280 Compliance to dietary intervention

281 Weight, serum β -OHB concentrations, additional anthropometric measurements that indicate
282 compliance to the dietary intervention and RMR are shown in **Table 2**. Both groups significantly reduced
283 weight (mean loss (%) -2.6; 95% CI -3.3, -1.9 and -2.9; -3.6, -2.1 for CER and IER, respectively), waist
284 circumference, BMI, %BF and energy intake demonstrating satisfactory compliance to the dietary
285 intervention. Compliance to fasting in the IER group was also satisfactory at endpoint as shown by the
286 significant increase in serum β -OHB after the 2-d SER compared to the non-SER period, and also the
287 baseline value (both comparisons $P = 0.001$). Serum β -OHB was significantly higher at endpoint following
288 CER compared with IER (non-SER, mean difference (mmol/L) 1.75; 95% CI 1.07, 2.86). Resting metabolic
289 rate did not differ significantly between groups at endpoint, however within the IER group there was a
290 significant decrease in RMR ($P = 0.006$). There were no significant differences between groups in the coping
291 strategies used to respond to stress, assessed by the COPE inventory (**Supplementary Figure 2**).

292 **Table 2.** Compliance to dietary intervention and other anthropometry measurements before and after following 4-week CER or IER diet.

	CER (N=22)			IER (N=21)			2-d SER	<i>P</i> value ² (2-d SER vs END)	Mean differences ³ between groups at END	<i>P</i> value ³ between groups at END
	Baseline	END	<i>P</i> value ¹ (Baseline vs END)	Baseline	END	<i>P</i> value ² (Baseline vs END)				
Weight (kg)	89.2 (80.1, 98.2)	86.2 (85.5, 86.9)	<0.001	87.7 (80.2, 95.2)	85.9 (85.2, 86.6)	<0.001	84.7 (77.5, 91.9)	0.003	0.36 (-0.62, 1.34)	0.464
Waist circumference (cm)^a	111 (104, 118)	105 (103, 106)	<0.001	107 (103, 111)	104 (103, 106)	<0.001	103 (98, 108)	0.432	1.01 (0.99, 1.02) [‡]	0.535
BMI (kg/m²)	31.0 (28.4, 33.5)	30.6 (30.4, 30.9)	<0.001	31.9 (29.8, 34.0)	30.5 (30.2, 30.7)	<0.001	30.8 (28.7, 32.9)	0.017	0.16 (-0.17, 0.49)	0.340
%BF	37.4 (34.1, 40.6)	36.4 (34.8, 37.9)	0.028	40.0 (37.2, 42.9)	37.4 (35.8, 39.0)	<0.001	38.7 (35.8, 41.5)	0.524	-1.0 (-3.2, 1.2)	0.357
Energy intake (kcal/d)	2140 (1833, 2446)	1264 (1091, 1436)	<0.001	2032 (1839, 2224)	1318 (1146, 1490)	<0.001	N/A	N/A	-55 (-299, 189)	0.651
Carbohydrates (g/d)	228 (190, 267)	147 (128, 165)	<0.001	230 (196, 267)	138 (120, 157)	<0.001	N/A	N/A	8.1 (-18.3, 34.6)	0.538

				264)						
Carbohydrates (%E)	40.0 (36.5, 43.6)	43.7 (41.4, 46.0)	0.027	42.0 (38.1, 46.0)	40.1 (37.8, 42.4)	0.340	N/A	N/A	3.6 (0.31, 6.90)	0.033
Protein (g/d)	91.6 (74.1, 109.1)	62.9 (55.0, 70.8)	<0.001	88.2 (76.2, 100.2)	72.6 (64.7, 80.5)	0.002	N/A	N/A	-9.7 (-20.9, 1.5)	0.087
Protein (%E)	16.1 (14.5, 17.7)	20.3 (18.3, 22.2)	<0.001	17.7 (15.3, 20.1)	22.3 (20.3, 24.3)	0.002	N/A	N/A	-2.1 (-4.89, 0.77)	0.149
Fat (g/d)	89.7 (72.9, 106.5)	46.1 (36.9, 55.3)	<0.001	86.0 (76.7, 95.3)	50.8 (41.5, 60.0)	<0.001	N/A	N/A	-4.7 (-17.8, 8.4)	0.472
Fat (%E)	37.4 (33.7, 41.0)	33.0 (30.3, 35.7)	0.009	38.2 (35.7, 40.7)	33.4 (30.7, 36.1)	0.001	N/A	N/A	-0.42 (-4.28, 3.43)	0.823
β-hydroxybutyrate (mmol/L)^a	0.09 (0.07, 0.12)	0.11 (0.08, 0.16)	0.164	0.07 (0.04, 0.11)	0.07 (0.05, 0.09)	0.748	0.20 (0.13, 0.31)	0.001	1.75 (1.07, 2.86) [¥]	0.004
RMR (kcal)	1380 (1242,	1356 (1300,	0.336	1401 (1279,	1325 (1266,	0.006	1304 (1183,	0.356	31 (-50, 112)	0.449

	1518)	1412)	1524)	1384)	1426)
293	CER, continuous energy restriction; IER, intermittent energy restriction; 2-d SER, measurements taken following 2-d severe energy restriction; END, measurements				
294	at endpoint (not following 2-d SER in case of IER group); % BF, percentage of body fat; BMI, body mass index; RMR, Resting Metabolic Rate; ¹ comparison within				
295	CER group by paired t-test; ² comparison within IER group by paired t-test; ³ comparison between groups at END by ANCOVA adjusted for baseline values and, for				
296	β -hydroxybutyrate only, also adjusted for % weight loss. Baseline results expressed as mean (95% CI) and END results expressed as estimated marginal means (95%				
297	CI) adjusted for baseline values and, for β -hydroxybutyrate, % weight loss, except ^a geometric means (95% CI). The differences between groups at END is expressed				
298	as mean differences (95% CI), except [¥] Exponents of mean differences in Ln values (the ratio of the geometric mean in CER to that in IER, with 95% CI of the				
299	geometric mean ratios).				

300 **Insulin sensitivity, glucose, lipids, adipokines and inflammatory markers**

301 Fasting markers of insulin sensitivity, lipids, adipokines and inflammation are presented in **Table 3**.
 302 Insulin sensitivity (R-QUICKI) significantly increased in the whole cohort when combining CER and IER (P
 303 <0.001), and insulin resistance (HOMA-IR), serum insulin and plasma glucose significantly decreased (P
 304 <0.005) (**Supplementary Table 1**), but there were no significant treatment effects on the magnitude of these
 305 changes (**Table 3**). Following IER, fasting plasma glucose was 4.6% higher (adjusted log ratio 95% CI 0.7,
 306 8.5, $P = 0.023$), and NEFA was 0.15 mmol/L lower (adjusted mean difference 95% CI -0.24, -0.06, $P =$
 307 0.002) compared with CER.

308 There were no significant treatment effects on fasting circulating concentrations of TAG, total
 309 cholesterol to HDL-cholesterol ratio, leptin, adiponectin, or the leptin to adiponectin ratio, the summary
 310 inflammatory score, nor on noradrenaline (normetanephrine) (**Table 3**). **Supplementary Table 2** shows the
 311 effects of treatment on individual inflammatory markers. Secondary analysis of baseline versus endpoint in
 312 the whole cohort (both groups combined) showed that there were decreases in fasting TAG ($P < 0.001$),
 313 insulin ($P = 0.005$), IL-1b ($P = 0.033$), leptin ($P < 0.001$) and adiponectin ($P = 0.008$) concentrations, total
 314 cholesterol: HDL-cholesterol ($P = 0.018$) and leptin:adiponectin ($P = 0.001$) ratios, and a significant increase
 315 in IL-1RA ($P = 0.007$) (**Supplementary Table 1**).

316 Further secondary analysis to investigate the acute effects of 2-d SER at endpoint showed that,
 317 relative to the non-SER endpoint in the IER group, 2 days of SER induced significant reductions in fasting
 318 HOMA-IR, serum insulin, TAG, leptin concentrations and leptin:adiponectin ratio, and TNF α concentrations
 319 (see **Supplementary table 2** for individual inflammation markers), and increased fasting serum NEFA
 320 concentrations within the IER group (**Table 3**). However, there were no acute effects of fasting on R-
 321 QUICKI, adiponectin, the summary inflammatory score, total cholesterol to HDL-cholesterol ratio or
 322 normetanadrenaline.

Table 3. Fasting insulin sensitivity, glucose, lipids, adipokines and inflammatory markers at baseline and following a 4-week continuous or intermittent energy restricted diet.

	CER (N=22)			IER (N=21)						<i>P</i> value between groups at END
	Baseline ^d	END ^b	<i>P</i> value	Baseline ^d	END ^b	<i>P</i> value	2-d SER ^d	<i>P</i> value (2-d SER vs END)	Mean differences between groups at END	
R-QUICKI ^c	0.39 (0.38, 0.41)	0.41 (0.40, 0.43)	0.013	0.39 (0.37, 0.42)	0.42 (0.40, 0.44)	0.006	0.41 (0.39, 0.44) ^a	0.810	-0.01 (-0.03, 0.02)	0.590
HOMA-IR ^{ac}	1.80 (1.50, 2.16)	1.50 (1.29, 1.75)	0.017	2.10 (1.65, 2.55)	1.68 (1.42, 1.98)	0.057	1.27 (0.97, 1.68)	0.008	0.89 (0.71, 1.12) [¥]	0.331
Glucose (mmol/L) ^a	4.86 (4.62, 5.11)	4.61 (4.48, 4.74)	0.001	4.88 (4.60, 5.18)	4.84 (4.69, 4.98) ^d	0.592	4.62 (4.39, 4.86)	0.058	0.95 (0.91, 0.99)[¥]	0.023
Insulin (mIU/L) ^{ac}	8.33 (6.98, 9.96)	7.32 (6.34, 8.44)	0.043	9.44 (7.62, 11.70)	7.84 (6.72, 9.14)	0.059	6.21 (4.72, 8.17)	0.011	0.93 (0.76, 1.15) [¥]	0.516
NEFA (mmol/L) ^c	0.55 (0.45, 0.64)	0.54 (0.48, 0.60)	0.985	0.51 (0.40, 0.62)	0.39 (0.33, 0.46)	0.015	0.59 (0.47, 0.71)	0.001	0.15 (0.06, 0.24)	0.001

	CER (N=22)			IER (N=21)			2-d SER ^d	<i>P</i> value (2-d SER vs END)	Mean differences between groups at END	<i>P</i> value between groups at END
	Baseline ^d	END ^b	<i>P</i> value	Baseline ^d	END ^b	<i>P</i> value				
TAG (mmol/L) ^{ac}	1.12 (0.90, 1.39)	0.96 (0.86, 1.06)	<0.001	1.38 (1.09, 1.75)	1.04 (0.93, 1.17)	0.018	0.98 (0.83, 1.14)	0.031	0.91 (0.78, 1.07) [¥]	0.270
Total cholesterol: HDL-cholesterol ratio ^{ac}	3.52 (3.17, 3.92)	3.44 (3.29, 3.60)	0.028	3.36 (3.01, 3.75)	3.51 (3.34, 3.68)	0.262	3.71 (3.40, 4.06)	0.171	0.98 (0.92, 1.05)	0.558
Leptin (µg/L) ^{ac}	18.0 (13.1, 24.7)	15.1 (13.5, 17.0)	0.009	23.5 (16.7, 33.0)	16.2 (14.3, 18.3)	0.001	14.4 (9.9, 20.9)	<0.001	0.94 (0.78, 1.11) [¥]	0.448
Adiponectin (mg/L) ^c	8.45 (6.62, 10.34)	7.76 (7.21, 8.32)	0.187	7.91 (5.89, 9.92)	7.31 (6.71, 7.90)	0.015	7.41 (5.56, 9.27)	0.613	0.46 (-0.36, 1.27)	0.267
Leptin:adiponectin ratio ^{ac}	2.40 (1.65, 3.49)	2.23 (1.96, 2.53)	0.020	3.53 (2.48, 5.02)	2.56 (2.23, 2.94)	0.024	2.34 (1.56, 3.52)	<0.001	0.87 (0.72, 1.05) [¥]	0.152
Inflammatory score ^{ef}	-0.03 (- 0.22, 0.16)	0.09 (-0.07, 0.24)	0.243	0.03 (-0.19, 0.25)	-0.11 (- 0.28, 0.06)	0.234	-0.02 (- 0.25, 0.20)	0.610	0.19 (-0.03, 0.42)	0.093
Normetadrenaline	489 (380, 563)	563 (428, 698)	0.406	491 (392, 590)	573 (427, 719)	0.255	538 (407, 669)	0.719	-9.0 (-209.1, 191.1)	0.928

	CER (N=22)			IER (N=21)						
	Baseline ^d	END ^b	<i>P</i> value	Baseline ^d	END ^b	<i>P</i> value	2-d SER ^d	<i>P</i> value (2-d SER vs END)	Mean differences between groups at END	<i>P</i> value between groups at END
	(pmol/L)									
	597)	699)		590)	719)		670)		191.0)	

325 CER, continuous energy restriction; IER, intermittent energy restriction; 2-d SER, measurements taken following 2-d severe energy restriction; END, measurements
 326 at endpoint (not following 2-d SER in case of IER group). Statistically significant values are in bold. ^a Geometric means with 95% CI; ^b Adjusted for baseline values
 327 and % weight loss; ^c 2 missing samples, IER n=19; ^d 1 missing sample, IER n=20; ^e 4 missing values, IER n=17, 1 missing value, CER n=20, due to technical
 328 challenges; ^f Summary inflammatory score: [z score(_{LN}TNF- α) + z score(_{LN}IL-6) + z score(_{LN}IL-8) + z score(_{LN}VEGF) + z score(IL-1B:IL-1FA ratio) + z
 329 score(_{LN}MCP)]. The differences between groups at END is expressed as mean difference (95% CI), except [¥] Exponents of mean differences in Ln values (the ratio of
 330 the geometric mean in CER to that in IER, with 95% CI of the geometric mean ratios).

331 **Blood pressure and heart rate variability**

332 There were no treatment effects on any of the ABP, IBI and HRV parameters over 24 h, day-time or
 333 night-time nor resting supine BP nor 30-minute resting supine IBI and HRV measurements (**Table 4**).
 334 However, there was a significant decrease in 24 h ($P < 0.01$), daytime ($P < 0.01$) and night time ($P < 0.05$)
 335 ambulatory DBP and MAP in the whole study cohort when comparing baseline to endpoint (**Supplementary**
 336 **Table 1**). Furthermore, there was a decrease in resting SBP ($P = 0.032$) and increase in 24 h IBI ($P = 0.045$)
 337 in the whole cohort regardless of treatment, but mean increases in daytime HRV in both groups combined
 338 did not reach statistical significance. No significant acute effects of a 2-d SER were found in any parameters
 339 within the IER group, except for resting DBP (mean difference in DBP: non-SER – 2-d SER 3.4 mm Hg
 340 (95% CI 0.8, 6.1), $P = 0.014$). Deviations from resting values for supine BP, average IBI, HR and HRV
 341 during the Stroop mental stress test were not different between CER and IER groups at endpoint relative to
 342 the baseline visit (data not shown).

343 **Table 4.** Blood pressure, heart rate variability and physical activity values before and after following a 4-week CER or IER diet.

	CER (N=22)			IER (N=21)				
	Baseline	END ^a	<i>P</i> value	Baseline	END ^a	<i>P</i> value	Mean differences between groups at END	<i>P</i> value between groups at END
24 h ambulatory measurements								
SBP (mm Hg) ^c	124 (119, 128)	121 (117, 124)	0.248	121 (116, 126)	119 (116, 123)	0.216	0.8 (-4.2, 5.9)	0.741
DBP (mm Hg) ^c	75 (72, 78)	72 (70, 74)	0.024	75 (72, 78)	71 (69, 74)	0.005	0.6 (-2.3, 3.4)	0.674
MAP (mm Hg) ^c	91 (88, 94)	88 (86, 91)	0.054	91 (87, 94)	88 (85, 90)	0.021	0.7 (-2.6, 4.0)	0.664
Activity (cpm) ^d	79 (72, 87)	81 (75, 87)	0.923	87 (75, 100)	82 (75, 88)	0.443	-0.8 (-9.5, 7.8)	0.847
Average IBI (ms) ^d	817 (780, 855)	823 (798, 849) ^b	0.122	789 (755, 824)	828 (798, 859) ^b	0.200	-5 (-45, 35) ^b	0.805
SDNN (ms) ^d	137 (123, 151)	145 (132, 158) ^b	0.069	137 (122, 152)	145 (129, 160) ^b	0.438	0.8 (-19.6, 21.1) ^b	0.938
RMSSD (ms) ^d	37.6 (28.1, 47.1)	40.4 (33.5, 47.3) ^b	0.562	41.7 (28.4, 55.0)	43.7 (35.6, 51.8) ^b	0.570	-3.3 (-14.0, 7.4) ^b	0.537
HF (ms ²) ^{dj}	336 (216, 523)	430 (326, 567) ^b	0.158	412 (241, 705)	454 (328, 628) ^b	0.451	0.95 (0.62, 1.46) ^{b, ¥}	0.800
Day-time ambulatory measurements								
SBP (mm Hg) ^c	127 (123, 131)	125 (121, 129)	0.521	126 (120, 131)	123 (119, 127)	0.228	2.1 (-3.4, 7.7)	0.438

	CER (N=22)			IER (N=21)			Mean differences between groups at END	P value between groups at END
	Baseline	END ^a	P value	Baseline	END ^a	P value		
DBP (mm Hg) ^c	78 (75, 81)	75 (73, 78)	0.069	79 (75, 82)	74 (72, 76)	0.003	1.1 (-1.9, 4.2)	0.448
MAP (mm Hg) ^c	94 (91, 97)	92 (90, 94)	0.149	94 (90, 98)	91 (88, 93)	0.018	1.5 (-2.0, 5.0)	0.394
Activity ^{8h} (cpm) ^e	113 (96, 130)	118 (107, 132)	0.501	114 (98, 130)	117 (105, 130)	0.626	0.9 (-15.8, 17.5)	0.916
Average IBI ^{8h} (ms) ^e	774 (738, 809)	760 (738, 781) ^b	0.812	733 (697, 769)	774 (750, 799) ^b	0.115	-15 (-48, 18) ^b	0.368
SDNN ^{8h} (ms) ^e	112 (100, 125)	113 (103, 123) ^b	0.252	103 (86, 119)	118 (107, 129) ^b	0.100	-4.8 (-19.9, 10.3) ^b	0.520
RMSSD ^{8h} (ms) ^e	33.7 (24.5, 43.0)	35.6 (29.2, 41.9) ^b	0.619	34.8 (23.7, 45.8)	39.0 (31.7, 46.2) ^b	0.395	-3.4 (-13.1, 6.3) ^b	0.480
HF ^{8h} (ms ²) ^e	264 (168, 416) ^j	403 (293, 513) ^{bk}	0.117	278 (153, 506) ⁱ	515 (389, 640) ^{bk}	0.230	-112 (-279, 55) ^{bk}	0.182
Night-time ambulatory measurements								
SBP (mm Hg) ^f	110 (104, 115)	104 (100, 108)	0.089	106 (99, 112)	105 (100, 109)	0.545	-0.3 (-6.4, 5.9)	0.928
DBP (mm Hg) ^f	65 (62, 69)	61 (58, 63)	0.026	61 (58, 65)	60 (57, 63)	0.202	0.5 (-3.5, 4.5)	0.796
MAP (mm Hg) ^f	80 (76, 84)	75 (72, 78)	0.039	76 (72, 80)	75 (72, 78)	0.291	0.3 (-4.0, 4.6)	0.895
Average IBI ^{5h} (ms) ^g	920 (852, 987)	965 (916, 1015)	0.102	949 (884, 1013)	956 (898, 1014)	0.508	9 (-67, 86)	0.801

	CER (N=22)			IER (N=21)				
	Baseline	END ^a	<i>P</i> value	Baseline	END ^a	<i>P</i> value	Mean differences between groups at END	<i>P</i> value between groups at END
SDNN ^{5h} (ms) ^g	76 (67, 87)	90 (81, 100)	0.028	82 (69, 98)	78 (67, 89)	0.473	12 (-3, 27)	0.106
RMSSD ^{5h} (ms) ^g	31.8 (23.0, 43.9)	47.7 (37.6, 57.9)	0.224	42.3 (31.0, 57.7)	48.4 (36.6, 60.2)	0.836	-0.6 (-16.3, 15.0)	0.934
HF ^{5h} (ms ²) ^g	400 (234, 683)	624 (456, 853)	0.024	579 (331, 1015)	553 (384, 797)	0.765	1.13 (0.70, 1.83) [¥]	0.613
Supine resting measurements (30 min)								
SBP (mm Hg) ^h	130 (123, 138)	123 (119, 127)	0.011	124 (117, 130)	123 (119, 127)	0.477	-0.4 (-5.8, 5.0)	0.889
DBP (mm Hg) ^h	79 (75, 84)	77 (74, 79)	0.248	77 (72, 82)	77 (74, 79)	0.677	0.2 (-3.4, 3.8)	0.916
Average IBI (ms) ⁱ	958 (921, 996)	972 (944, 1001)	0.099	917 (866, 967)	983 (953, 1013)	0.018	-11 (-53, 32)	0.609
Heart rate (bpm) ⁱ	63.1 (60.4, 65.9)	62.3 (60.5, 64.1)	0.101	66.2 (62.7, 69.8)	61.3 (59.3, 63.2)	0.013	1.0 (-1.7, 3.8)	0.451
RMSSD (ms) ^{ij}	35.1 (27.5, 44.8)	32.4 (27.2, 38.6)	0.811	29.5 (24.3, 35.9)	38.7 (32.1, 46.5)	0.117	0.84 (0.65, 1.08) [¥]	0.169
HF (ms ²) ^{ij}	390 (227, 670)	350 (260, 471)	0.951	291 (199, 427)	450 (330, 615)	0.104	0.78 (0.50, 1.20) [¥]	0.247

344 CER, continuous energy restriction; IER, intermittent energy restriction; END, measurements at endpoint (not following 2-d SER in case of IER group); SBP,

345 systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; IBI, interbeat interval; bpm, beats per minute; SDNN, standard deviation of all

346 NN intervals; ms, milliseconds; RMSSD, the square root of the mean of the sum of squares of differences between adjacent NN intervals; HF, high frequency power.
 347 Statistically significant values are in bold. No significant differences between the 2-d SER and non-SER endpoints were found in any parameter within the IER
 348 group (paired T-test or Wilcoxon signed rank test for related samples), except for resting DBP (mean difference non-SER – 2-d SER 3.4 mm Hg (95% CI 0.8, 6.1), P
 349 = 0.014). ^a Adjusted for baseline values and % weight loss; ^b Also adjusted for activity; ^c IER group n=19, CER group n=20; ^d IER group n=16, CER group n=22; ^e
 350 IER group n=17, CER group n=22; ^f IER group n=18, CER group n=20; ^g IER group n=14, CER group n=19; ^h IER group n=21, CER group n=21; ⁱ IER group
 351 n=19, CER group n=21; ^j Geometric means with 95% CI; ^k Adjusted for baseline values as LN values, due to deviation from normal distribution at baseline only. The
 352 differences between groups at END is expressed as mean difference (95% CI), except [¥] Exponents of mean differences in Ln values (the ratio of the geometric mean
 353 in CER to that in IER, with 95% CI of the geometric mean ratios).

354 DISCUSSION

355 The results of this 4-week randomized controlled trial in men and women with central obesity does
 356 not support the primary hypothesis that IER would acutely increase insulin sensitivity to a greater extent than
 357 CER when weight loss is equivalent. Previous randomized controlled trials reported greater increases in
 358 insulin sensitivity and decreases in fasting insulin in overweight women following IER diets compared to
 359 CER diets for 3 months or 6 months despite equivalent weight loss [29,30]. Here, we hypothesised that acute
 360 fluctuations in fuel oxidation and ketogenesis might be involved in short-term improvements in metabolic
 361 function, but the results of this study demonstrate that a longer intervention duration is necessary to effect
 362 changes in fasting insulin sensitivity measures [29,30].

363 Despite the lack of effect on fasting insulin/insulin resistance, there was a significant, but small,
 364 reduction in fasting glucose concentrations following CER that was not observed following IER. Although
 365 Harvie *et al.*, reported greater decreases from baseline in fasting glucose following IER compared to CER in
 366 premenopausal overweight women (duration 6 months) [29], other studies have been unable to show this in
 367 various populations using a diverse range of IER protocols [28,30,50–54]. Antoni *et al.* (2018) compared the
 368 effects of achieving 5% weight loss (over varying intervention durations) by either IER or CER on fasting
 369 glucose, and although there were no differences between groups at endpoint, there was a significant within-
 370 group increase in fasting glucose concentration following IER [50].

371 Antoni *et al.* (2018) also showed a trend towards a within-group reduction in fasting NEFA
 372 following IER, although there were no statistically significant differences between groups [50]. This is
 373 consistent with the current observation of reduced fasting NEFA concentrations following IER compared to
 374 CER. A reduction in fasting NEFA concentrations either suggests reduced activity of hormone sensitive
 375 lipase (HSL), which lipolyses TAG in adipose tissue to release NEFA and glycerol into the circulation
 376 during fasting periods, or a greater uptake of circulating NEFA by the liver, heart and skeletal muscle for
 377 fatty acid oxidation and ketogenesis [55]. HSL activity is predominantly regulated by insulin [56], but in this
 378 case there were no differences between groups in fasting insulin in the current study and so reduced HSL
 379 activity is an unlikely reason for the differences observed in fasting NEFA concentrations. Therefore, a
 380 suppression of fasting NEFA, together with a lack of reduction in fasting glucose plasma concentrations
 381 suggests that the 4-week IER regime induced adaptive shifts in energy metabolism. A 2-week intermittent
 382 fasting regime, calculated to be isocaloric with the control diet and to avoid weight loss (albeit alternate day

383 fasting rather than 5:2 pattern), resulted in greater phosphorylation of glycogen synthase kinase in muscle
 384 tissue taken from normal-weight, healthy men, suggesting adaptation that favoured glycogenesis [57]. In
 385 order to disentangle the complex, adaptive metabolic changes that occur as a result of repeated periods of
 386 IER, fasting and postprandial changes in glucose, C-peptide, insulin, glucagon, glycerol and NEFA should be
 387 tracked on a daily basis over at least 2 weeks in future studies.

388 Leptin and adiponectin are both hormones secreted by adipocytes. Both adipokines are implicated in
 389 the pathogenesis of cardiometabolic diseases mediated by excess intra-abdominal fat, although leptin
 390 concentrations are elevated in obesity due to leptin resistance, associated with pro-inflammatory/pro-
 391 atherogenic pathways, whereas adiponectin is reduced in visceral obesity and associated with insulin-
 392 sensitising and anti-inflammatory properties [58]. The current results show that plasma fasting leptin
 393 decreased markedly following both diets. Adiponectin decreased slightly following IER only, although there
 394 were no between-group differences. This is comparable to a previous study where significant decreases in
 395 leptin were found to be independent of the type of energy restriction followed [29]. Reduced leptin observed
 396 in both treatment groups reflects the comparable reduction in fat mass presented by both groups [59].
 397 Klempel *et al.* showed that both adiponectin and leptin significantly decreased after two different intermittent
 398 fasting approaches [60]. Regarding acute effects, leptin was further decreased following the 2-d SER relative
 399 to the non-2-d SER endpoint, whereas adiponectin was not acutely affected. The results of this study are
 400 consistent with previous reports that <5% body weight loss appears to have very little effect on adiponectin
 401 concentrations [61], and that adiponectin is likely to increase with larger changes in body weight over longer
 402 periods of time [29,61].

403 There is compelling evidence that suggests an increase in leptin, with the activation of the brain
 404 melanocortin system, links obesity with overactivation of sympathetic renal activity and elevated BP [62]. A
 405 decrease in leptin may be one of the mechanisms responsible for the BP lowering-effects of weight loss.
 406 Therefore, in the light of the equivalence observed between diets in reducing leptin, and the lack of effect of
 407 both diets in reducing inflammation and markers of sympathetic activity (normetadrenaline, HRV), then it is
 408 not surprising that there were no superior effects of following an IER diet compared to CER on ambulatory
 409 BP. Studies investigating the effects of intermittent fasting on BP in humans are scarce and have only
 410 reported resting office BP, whereas the current study assessed both resting supine and 24 h ambulatory BP.
 411 Previous reports have demonstrated significant reductions in resting SBP and/or DBP independent of type of

weight loss diet, which is in line with the supine resting BP data from the current study [30,52,63], although another study showed greater reductions in resting office SBP following IER compared to CER [50]. The studies assessing HRV in intermittent fasting are limited and restricted to animal models. Mager *et al.* measured 24 h HRV in rats maintained on IER (alternate day feeding) or CER (40% energy reduction) and showed an increase in high frequency power (representative of increased parasympathetic activity) that was comparable between the two diets, with maximal effects achieved after 4 to 5 weeks [64]. Animal studies are highly controlled and there is no risk of non-compliance to dietary intervention. An insufficient degree of energy restriction during fast days may be a potential explanation for the lack of effect in the current study. It might also be the case that this subject population, although metabolically at-risk, were not sufficiently compromised by increased sympathetic activity at baseline in order to induce a measurable increase in HRV.

The effect of central adiposity on insulin resistance is thought to be mediated partly via chronic low-grade inflammation [65]. Therefore, IER may confer a greater increase in insulin sensitivity relative to CER [29,30] by acutely modulating production of systemic pro-inflammatory cytokines (IL-6, IL-8, TNF- α , IL-1 β , MCP-1), the IL-1 receptor antagonist, IL-1Ra, all of which have been shown to be correlated with insulin resistance [66–70], and the angiogenic growth factor VEGF which is associated with visceral fat and other components of the metabolic syndrome [71]. The lack of effect of either diet on a summary score of low-grade inflammation, despite weight loss and reductions in waist circumference (indicating reductions in intra-abdominal adiposity), may be related to the basal low burden of inflammation in this mainly healthy population. One study previously reported decreased TNF- α in overweight adults with asthma following 8 weeks of alternate day fasting, although there was no control group so this change cannot be attributed to the pattern of energy restriction [72]. Nevertheless, overweight adults with asthma are likely to have a greater burden of chronic low-grade inflammation compared to overweight adults with no overt medical conditions, as indicated by the lack of effect on IL-6 and TNF- α in healthy populations with obesity following alternate day fasting (comparing before and after) [73], or on IL-6, TNF- α and CRP following a 5:2 pattern fasting relative to CER [29,30].

Despite the higher fasting glucose concentrations following IER relative to CER, a difference which, although statistically significant, is unlikely to be clinically significant, the findings from this study generally support the use of IER in the short-term for weight loss and cardiometabolic benefit as an equally effective alternative to daily energy restriction. Caution should be taken over longer periods as the use of meal

441 replacements for the 2-d period of severe energy restriction could lead to reduced weekly intakes of heart-
 442 healthy foods such as whole fruits and vegetables and whole grains relative to a continuous healthy eating,
 443 energy restricted diet. The study was strengthened by the fact that ambulatory measurements were taken for
 444 BP, HR and HRV, thereby avoiding “white-coat hypertension” effects and facilitating the presentation of
 445 free-living average values [74]. The low attrition rates during the trial avoided any bias from differential
 446 drop-out rates between treatments. A large array of mechanistic measurements were made to allow a greater
 447 insight into the short-term adaptive cardiometabolic changes that occur in the early stages of following a 5:2
 448 pattern (with 2 consecutive SER days) that could explain any differential effects on components of the
 449 metabolic syndrome, such as insulin sensitivity as previously observed in women [29,30].

450 Although the relatively large number of outcomes could have increased the risk of type 1 errors, in
 451 fact this is unlikely as there were only statistically significant treatment effects on fasting glucose and NEFA,
 452 which are consistent with changes in fuel utilisation as a result of intermittent SER. Other limitations of the
 453 study include the fact that insulin sensitivity was assessed in the fasting state only. Gold standard methods
 454 for measuring insulin sensitivity, such as the hyperinsulinaemic euglycaemic clamp or the frequently
 455 sampled intravenous glucose tolerance test, are challenging to carry out and impose a greater degree of
 456 burden upon participants. Nevertheless, the lack of any postprandial measures of insulin sensitivity, which
 457 may have revealed adaptive differences in glucose homeostasis following an oral glucose tolerance test, is an
 458 importance factor to consider before dismissing the possibility that there may be a greater degree of
 459 improvement in insulin sensitivity in the first weeks following the commencement of a 5:2 diet with
 460 consecutive fasting days. In addition, the fact that HRV and ABP measurements were made simultaneously
 461 (necessitating the hourly inflation/deflation of the ABP cuff throughout the night) could have interfered with
 462 night-time HRV measurements by disrupting normal sleep patterns. Furthermore, although the study
 463 population had waist circumference measurements associated with high risk of cardiometabolic diseases, the
 464 current findings cannot be extrapolated to a more high-risk population such as subjects with pre-diabetes or
 465 hypertension.

466 In conclusion, a 4-week period of IER induces short-term metabolic adaptations that favour
 467 increased hepatic glucose output and greater efficiency of fatty acid utilisation during the post-absorptive
 468 state. A greater uptake of NEFA by skeletal muscle, liver and heart, resulting in increased fatty acid
 469 oxidation (involved in the concept “metabolic flexibility”) [55], would be a beneficial adaptation to short

470 periods of SER that would reduce accumulation of intramyocellular TAG and improve insulin signalling in
471 skeletal muscle. Whether this effect is a transient adaptation to IER or whether it can persist over longer
472 periods of IER is not clear and requires confirmation in studies of 6 months to 1-year duration. It is possible
473 that larger amounts of weight loss and consequent improvements in metabolic function that would occur over
474 longer periods might outweigh the subtle shifts in glucose and fatty acid metabolism observed here.
475 Components of the metabolic syndrome, such as insulin resistance, blood pressure, and lipids, as well as
476 leptin, were all reduced by energy restricted diets, regardless of the weekly distribution of energy intake. The
477 key message from the results of this trial is that most of the changes in markers of cardiometabolic health
478 that occurred after 4 weeks were similar following both diets. The most logical inference is that loss of fat
479 mass is the primary driver of improvements in insulin sensitivity and other cardiometabolic health markers,
480 and that moderate fluctuations in ketogenesis did not demonstrably modify these changes.

481 **Conflict of interest**

482 The authors have nothing to disclose.

483

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491

492 **Authors' contributions to the manuscript**

493 AMP and WLH designed the research, performed statistical analysis and wrote the paper. AMP, CB, LPB,
494 CK, PCK, IMDA and EJJ conducted the study and analysed data. WLH had primary responsibility for the
495 final content of the manuscript. All authors read and approved the final manuscript.

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710

1 **Supplementary Figure 1. Study timeline.**

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10 **Supplementary Figure 2. COPE inventory.**

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Whole cohort			
	Baseline	END	<i>P</i> value ¹
Weight (kg)	89.5 (82.8, 96.2)	86.9 (80.3, 93.4)	<0.001
Waist circumference (cm) ^a	109 (104, 113)	105 (100, 110)	<0.001
BMI (kg/m²)	31.9 (30.1, 33.8)	31.0 (29.1, 32.8)	<0.001
%BF	39.7 (37.4, 42.0)	37.7 (34.7, 40.6)	0.002
Seven-day energy intake (kcal/d)	2035 (1852, 2218)	1287 (1136, 1439)	<0.001
β-hydroxybutyrate (mmol/L) ^a	0.07 (0.05, 0.10)	0.09 (0.07, 0.12)	0.467
RMR (kcal)	1371 (1266, 1477)	1328 (1237, 1418)	0.019
R-QUICKI	0.39 (0.38, 0.41)	0.42 (0.40, 0.43)	<0.001
HOMA-IR ^a	1.90 (1.64, 2.21)	1.59 (1.32, 1.92)	0.002
Glucose (mmol/L) ^a	4.82 (4.62, 5.02)	4.69 (4.49, 4.90)	0.004
Insulin (mIU/L) ^a	8.87 (7.66, 10.28)	7.62 (6.40, 9.07)	0.005
NEFA (mmol/L)	0.52 (0.44, 0.59)	0.47 (0.40, 0.53)	0.060
Triglycerides (mmol/L) ^a	1.23 (1.03, 1.48)	1.01 (0.86, 1.18)	<0.001
Total cholesterol: HDL cholesterol ratio ^a	3.64 (3.36, 3.95)	3.54 (3.26, 3.84)	0.018
Leptin (μg/L) ^a	22.7 (17.8, 28.9)	16.8 (12.6, 22.3)	<0.001
Adiponectin (mg/L)	8.46 (7.06, 9.86)	7.68 (6.49, 8.88)	0.008
Leptin:adiponectin ratio ^a	3.08 (2.35, 4.02)	2.50 (1.85, 3.37)	0.001
Inflammatory score ^d	0.00 (-0.14, 0.14)	-0.02 (-0.16, 0.12)	1.000
TNFα (ng/L) ^b	0.83 (0.67, 0.96)	0.68 (0.58, 0.89)	0.007 ^c
Plasma IL-6 (ng/L) ^b	0.78 (0.55, 1.18)	0.74 (0.52, 1.03)	0.282 ^c
Plasma IL-8 (ng/L) ^a	2.38 (1.98, 2.87)	2.21 (1.87, 2.60)	0.336

VEGF (ng/L) ^a	19.2 (13.6, 27.0)	15.8 (11.5, 21.8)	0.081
IL-1b (ng/L) ^b	0.76 (0.56, 1.12)	0.67 (0.54, 0.83)	0.040 ^c
MCP-1 (ng/L)	48.2 (41.7, 54.7)	44.0 (37.7, 50.3)	0.133
IL-1RA (ng/L) ^a	63.6 (48.7, 82.9)	86.0 (66.7, 110.8)	0.008
Normetadrenaline (pmol/L)	474 (396, 551)	601 (485, 718)	0.159
24 h ambulatory measurements			
SBP (mm Hg)	124 (119, 128)	120 (117, 123)	0.087
DBP (mm Hg)	75 (72, 78)	72 (70, 74)	<0.001
MAP (mm Hg)	91 (89, 93)	88 (86, 90)	0.002
Activity (cpm)	79 (72, 87)	80.1 (75.8, 85.8)	0.631
Average IBI (ms)	817 (780, 855)	822 (795, 849)	0.048
SDNN (ms)	137 (123, 151)	145 (133, 157)	0.123
RMSSD (ms)	37.6 (28.1, 47.1)	41.8 (35.3, 48.2)	0.156
HF (ms ²) ^a	336 (216, 523)	440 (333, 581)	0.127
Day-time ambulatory measurements			
SBP (mm Hg)	127 (123, 131)	124 (121, 127)	0.185
DBP (mm Hg)	78 (75, 81)	75 (73, 77)	0.001
MAP (mm Hg)	94 (92, 96)	91 (89, 93)	0.007
Activity ^{8h} (cpm)	113 (96, 130)	118 (109, 127)	0.399
Average IBI ^{8h} (ms)	774 (738, 809)	766 (741, 789)	0.242
SDNN ^{8h} (ms)	112 (100, 125)	115 (105, 124)	0.077
RMSSD ^{8h} (ms)	33.7 (24.5, 43.0)	37.3 (31.6, 42.9)	0.115
HF ^{8h} (ms ²)	264 (168, 416)	450 (343, 557)	0.052
Night-time ambulatory measurements			

SBP (mm Hg)	110 (104, 115)	104 (101, 108)	0.102
DBP (mm Hg)	65 (62, 69)	60 (58, 63)	0.011
MAP (mm Hg)	78 (75, 81)	75 (73, 77)	0.022
Average IBI ^{5h} (ms)	920 (852, 987)	957 (916, 1001)	0.125
SDNN ^{5h} (ms) ^a	76 (67, 87)	83.8 (76.3, 92.0)	0.393
RMSSD ^{5h} (ms)	31.8 (23.0, 43.9)	48.8 (40.5, 57.0)	0.110
HF ^{5h} (ms ²) ^a	400 (234, 683)	599 (449, 800)	0.091
Supine resting measurements (30 min)			
SBP (mm Hg)	130 (123, 138)	124 (120, 128)	0.032
DBP (mm Hg)	79 (75, 84)	77 (74, 80)	0.283
Average IBI (ms)	958 (921, 996)	978 (951, 1006)	0.004
Heart rate (bpm)	63.1 (60.4, 65.9)	61.8 (60.0, 63.6)	0.003
RMSSD (ms) ^a	35.1 (27.5, 44.8)	36.0 (30.4, 42.7)	0.209
HF (ms ²) ^a	390 (227, 670)	413 (295, 577)	0.184

14 END, measurements at endpoint (not following 2-d SER in case of IER group); % BF, percentage of body
15 fat; BMI, body mass index; RMR, Resting Metabolic Rate; SBP, systolic blood pressure; DBP, diastolic
16 blood pressure; MAP, mean arterial pressure; IBI, interbeat interval; bpm, beats per minute; SDNN, standard
17 deviation of all NN intervals; ms, milliseconds; RMSSD, the square root of the mean of the sum of squares
18 of differences between adjacent NN intervals; HF, high frequency power. Comparison within whole cohort
19 by paired T-test or Wilcoxon signed rank test for related samples. Statistically significant values are in bold.^a
20 Geometric means with 95% CI; ^b Median (lower and upper IQR); ^c *p* value obtained using related samples
21 Wilcoxon Signed Rank Test; ^d Summary inflammatory score: [z score(_{LN}TNF-α) + z score(_{LN}IL-6) + z
22 score(_{LN}IL-8) + z score(_{LN}VEGF) + z score(IL-1B:IL-1FA ratio) + z score(_{LN}MCP).

	CER (N=22)		Within group: Baseline vs END	IER (N=21)		Within group: Baseline vs END		Within group: END vs SER	Differences between groups at END	
	Baseline	END	<i>p</i> value ¹	Baseline ^c	END ^c	<i>p</i> value ²	2-d SER ^d	<i>p</i> value ²	Mean differences ³	<i>p</i> value ³
TNFα (ng/L) ^b	0.78 (0.62, 0.89)	0.68 (0.58, 0.85)	0.054 ^e	0.89 (0.74, 1.01)	0.73 (0.58, 0.90)	0.038^e	0.68 (0.58, 0.84)	<0.001^e	-	0.697 [±]
Plasma IL-6 (ng/L) ^b	0.63 (0.53, 0.88)	0.65 (0.49, 0.96)	0.903 ^e	0.94 (0.60, 1.50)	0.77 (0.63, 1.12)	0.147 ^e	0.88 (0.73, 1.19)	0.354 ^e	-	0.224 [±]
Plasma IL-8 (ng/L) ^a	2.17 (1.76, 2.68)	2.10 (1.73, 2.53)	0.672	2.47 (1.74, 3.51)	2.08 (1.69, 2.55)	0.366	2.36 (1.68, 3.33)	0.654	1.01 (0.76, 1.34) [¥]	0.948
VEGF (ng/L) ^a	25.2 (16.0, 39.8)	19.4 (14.8, 25.5)	0.477	14.3 (8.7, 23.4)	12.6 (9.5, 16.8)	0.104	12.3 (7.8, 19.3)	0.469	1.54 (1.03, 2.30)[¥]	0.038
IL-1b (ng/L) ^b	0.74 (0.59, 0.95)	0.67 (0.57, 0.83)	0.211 ^e	0.79 (0.55, 1.16)	0.64 (0.51, 0.89)	0.117 ^e	0.65 (0.57, 0.81)	0.809 ^e	-	0.522 [±]
MCP-1 (ng/L)	49.6 (39.6, 59.5)	46.1 (39.1, 53.1)	0.580	48.3 (39.2, 57.4)	41.5 (34.0, 49.1)	0.127	45.6 (29.8, 61.4)	0.576	4.5 (-5.8, 14.9)	0.379

IL-1RA (ng/L) ^a	65.6 (47.9, 89.7)	79.7 (62.1, 102.2)	0.013	62.9 (39.8, 99.4)	84.9 (64.9, 111.0)	0.106	88.5 (64.6, 121.3)	0.319	0.94 (0.65, 1.36) [¥]	0.731
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24 CER, continuous energy restriction; IER, intermittent energy restriction; IL, interleukin; 2-d SER, measurements taken following 2-d severe energy restriction; END,
25 measurements at endpoint (not following 2-d SER in case of IER group). ¹ comparison within CER group by paired t-test; ² comparison within IER group by paired
26 t-test; ³ comparison between groups at END by ANCOVA adjusted for baseline values and percentage weight loss. Baseline results expressed as mean (95% CI) and
27 END results expressed as estimated marginal means (95% CI) adjusted for baseline values and percentage weight loss, except ^a geometric mean (95% CI) and ^b
28 median (lower and upper IQR); ^c 2 missing samples in IER group, n=19; ^d 1 missing value, IER n=20; ^e *p* value obtained using related samples Wilcoxon Signed
29 Rank Test. The differences between groups at END is expressed as mean difference (95% CI), except [¥] Exponents of mean differences in Ln values (the ratio of the
30 geometric mean in CER to that in IER, with 95% CI of the geometric mean ratios); ± Use of Mann–Whitney U test where data remained not normally distributed
31 following LN transformation; there were no differences between groups.