DOI:
10.1016/j.clnu.2019.07.014

Document Version
Peer reviewed version

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

28th June 2019

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ABSTRACT

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

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

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

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

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

Abbreviations

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

INTRODUCTION

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

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

MATERIAL AND METHODS

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

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

Study protocol

The Met-IER study (The Impact Of An Intermittent Energy Restricted Diet On Insulin Sensitivity In Men and Women With Central Obesity), was a 4-week parallel arm randomized controlled trial designed to compare the relative cardiometabolic effects of short-term IER and CER in men and women with central obesity. The study was conducted at the metabolic research unit (MRU) at King's College London, United Kingdom, between February and July 2016. Potential participants attended a screening visit upon which anthropometric and resting blood pressure measurements were taken, alongside a fasting blood sample to assess lipid profile, glucose, liver function and haematology to confirm eligibility to take part in the study. Before randomisation to treatment, eligible participants were asked to complete a 7-day food record and a validated eating habits questionnaire (Dutch Eating Behaviour Questionnaire, DEBQ), which evaluates dietary restraint and emotional/external eating [34]. The DEBQ was used to assess disordered eating behaviour and to check that the groups were balanced for eating styles. Treatment was randomly allocated by the lead researcher using a computer online MinimPy 0.3 (Copyright (c) 2011 Mahmoud Saghaei, http://minimpy.sourceforge.net) by minimization for sex, BMI, ethnicity and waist circumference. Participants attended the MRU in the fasted state for baseline measurements of body composition, resting blood pressure, resting and post-mental stress HRV, resting metabolic rate (RMR), to provide fasting blood samples, and to be fitted with 24 h ambulatory blood pressure (ABP) and HRV monitors; participants also completed the COPE inventory [35], designed to assess coping strategies to respond to stress, and performed a Mnemonic Similarity Task (MST) [36]. The MST was carried out as a collaboration with the Institute of Psychiatry (IoP) at King's College London (Denmark Hill campus) as an exploratory investigation into 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 O_2 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 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

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

Body weight and composition

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

Clinical and laboratory parameters

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

HS X biochip; Randox Laboratories Limited, County Antrim). Plasma free normetanephrine (NMN), the stable O-methylated metabolite of norepinephrine, was measured as an additional marker of sympathetic activity by liquid chromatography-tandem mass spectrometry using TurboFlowTM, an online sample preparation system. Insulin sensitivity was assessed by the revised quantitative insulin sensitivity check index (RQUICKI: 1/(log glucose (mg/dL) + logInsulin (μU/mL) + logFFA (mmol/L)))[42], and insulin resistance by the homeostatic model assessment (HOMA-IR: Glucose mM x Insulin mU/L / 22.5).

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

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 score($_{LN}$MCP)

Blood pressure and heart rate variability measurements

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

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

Statistical analysis

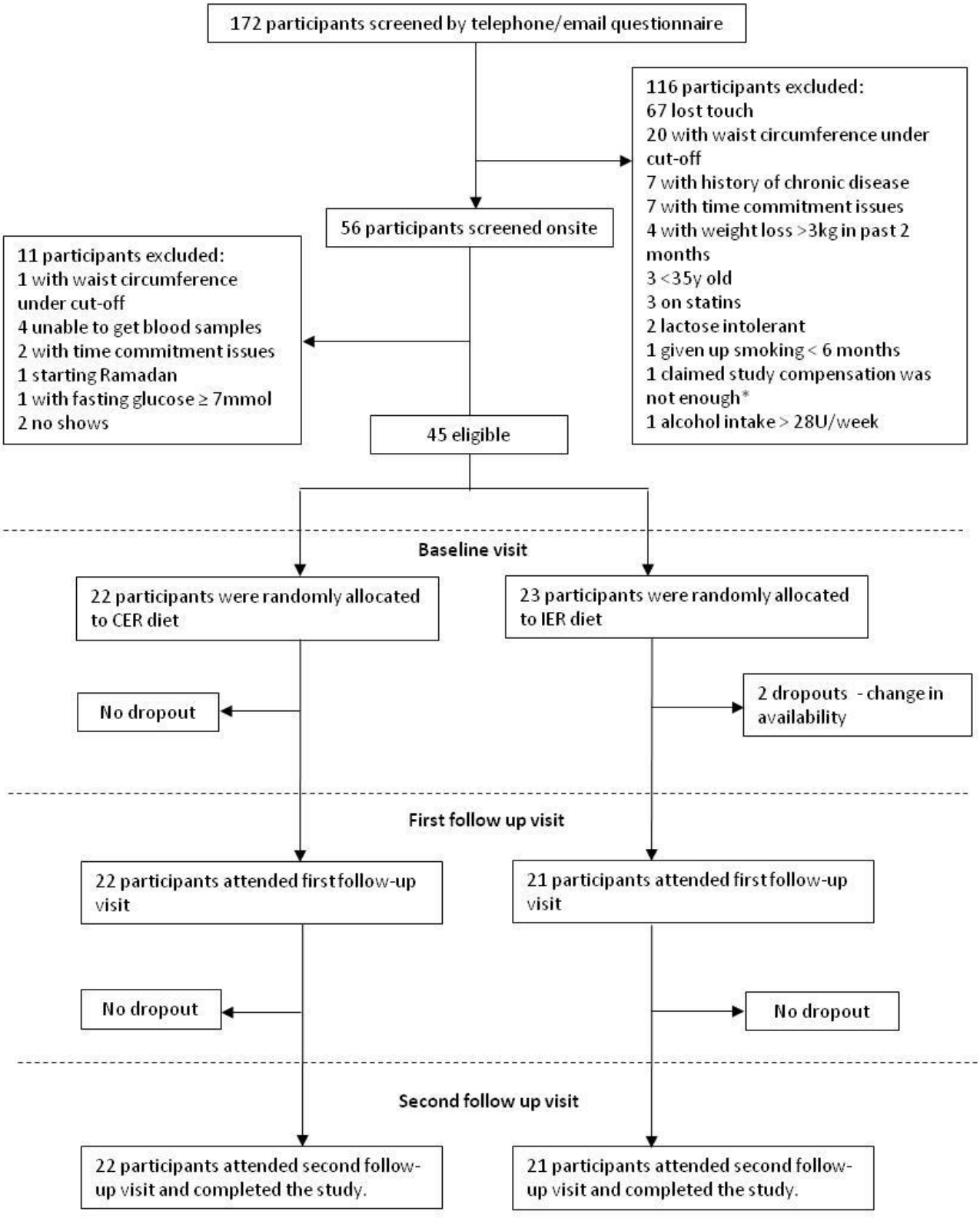
The primary endpoint of the study was R-QUICKI, a simple fasting index of insulin sensitivity that has previously been demonstrated to correlate highly with results from the euglycaemic hyperinsulinaemic clamp in lean and impaired glucose tolerance subjects [48]. Expected changes in R-QUICKI were therefore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

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

RESULTS

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

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



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

General characteristics at screening of the completers are reported in **Table 1**. Subjects were predominantly pre-hypertensive, with an average BMI of 31 kg/m^2, 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/m^2)	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)

Compliance to dietary intervention

Weight, serum β-OHB concentrations, additional anthropometric measurements that indicate compliance to the dietary intervention and RMR are shown in **Table 2**. Both groups significantly reduced weight (mean loss (%) -2.6; 95% CI -3.3, -1.9 and -2.9; -3.6, -2.1 for CER and IER, respectively), waist circumference, BMI, %BF and energy intake demonstrating satisfactory compliance to the dietary intervention. Compliance to fasting in the IER group was also satisfactory at endpoint as shown by the significant increase in serum β-OHB after the 2-d SER compared to the non-SER period, and also the baseline value (both comparisons $P = 0.001$). Serum β-OHB was significantly higher at endpoint following CER compared with IER (non-SER, mean difference (mmol/L) 1.75; 95% CI 1.07, 2.86). Resting metabolic rate did not differ significantly between groups at endpoint, however within the IER group there was a significant decrease in RMR ($P = 0.006$). There were no significant differences between groups in the coping 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)**						
	Baseline	**END**	***P* value [1] (Baseline vs END)**	**Baseline**	**END**	***P* value [2] (Baseline vs END)**	**2-d SER**	***P* value [2] (2-d SER vs END)**	**Mean differences[3] between groups at END**	***P* value[3] between groups at 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/m^2)	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,	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)

CER, continuous energy restriction; IER, intermittent energy restriction; 2-d SER, measurements taken following 2-d severe energy restriction; END, measurements 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 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 β-hydroxybutyrate only, also adjusted for % weight loss. Baseline results expressed as mean (95% CI) and END results expressed as estimated marginal means (95% 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 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 geometric mean ratios).

Insulin sensitivity, glucose, lipids, adipokines and inflammatory markers

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

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

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

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

	CER (N=22)			IER (N=21)						
	Baseline[d]	**END[b]**	***P* value**	**Baseline[d]**	**END[b]**	***P* value**	**2-d SER[d]**	***P* value (2-d SER vs END)**	**Mean differences between groups at END**	***P* value 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)						
	Baseline[d]	**END[b]**	***P* value**	**Baseline[d]**	**END[b]**	***P* value**	**2-d SER[d]**	***P* value (2-d SER vs END)**	**Mean differences between groups at END**	***P* value between groups at END**
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 (428,	0.406	491 (392,	573 (427,	0.255	538 (407,	0.719	-9.0 (-209.1,	0.928

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

CER, continuous energy restriction; IER, intermittent energy restriction; 2-d SER, measurements taken following 2-d severe energy restriction; END, measurements 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 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 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 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 the geometric mean in CER to that in IER, with 95% CI of the geometric mean ratios).

Blood pressure and heart rate variability

There were no treatment effects on any of the ABP, IBI and HRV parameters over 24 h, day-time or night-time nor resting supine BP nor 30-minute resting supine IBI and HRV measurements (**Table 4**). However, there was a significant decrease in 24 h ($P < 0.01$), daytime ($P < 0.01$) and night time ($P < 0.05$) ambulatory DBP and MAP in the whole study cohort when comparing baseline to endpoint (**Supplementary Table 1**). Furthermore, there was a decrease in resting SBP ($P = 0.032$) and increase in 24 h IBI ($P = 0.045$) in the whole cohort regardless of treatment, but mean increases in daytime HRV in both groups combined did not reach statistical significance. No significant acute effects of a 2-d SER were found in any parameters within the IER group, except for resting DBP (mean difference in DBP: non-SER – 2-d SER 3.4 mm Hg (95% CI 0.8, 6.1), $P = 0.014$). Deviations from resting values for supine BP, average IBI, HR and HRV during the Stroop mental stress test were not different between CER and IER groups at endpoint relative to 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]**	***P* value**	**Baseline**	**END[a]**	***P* value**	**Mean differences between groups at END**	***P* 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)				
	Baseline	**END[a]**	***P* value**	**Baseline**	**END[a]**	***P* value**	**Mean differences between groups at END**	***P* value between groups at END**
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]**	***P* value**	**Baseline**	**END[a]**	***P* value**	**Mean differences between groups at END**	***P* value between groups at END**
SDNN[5h] (ms) [gj]	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) [gj]	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

CER, continuous energy restriction; IER, intermittent energy restriction; END, measurements at endpoint (not following 2-d SER in case of IER group); SBP, systolic blood pressure; DBP, diastolic bood pressure; MAP, mean arterial pressure; IBI, interbeat interval; bpm, beats per minute; SDNN, standard deviation of all

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. 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 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* = 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] 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 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 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 in CER to that in IER, with 95% CI of the geometric mean ratios).

DISCUSSION

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

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

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

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

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

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

412 weight loss diet, which is in line with the supine resting BP data from the current study [30,52,63], although
413 another study showed greater reductions in resting office SBP following IER compared to CER [50]. The
414 studies assessing HRV in intermittent fasting are limited and restricted to animal models. Mager *et al*.
415 measured 24 h HRV in rats maintained on IER (alternate day feeding) or CER (40% energy reduction) and
416 showed an increase in high frequency power (representative of increased parasympathetic activity) that was
417 comparable between the two diets, with maximal effects achieved after 4 to 5 weeks [64]. Animal studies are
418 highly controlled and there is no risk of non-compliance to dietary intervention. An insufficient degree of
419 energy restriction during fast days may be a potential explanation for the lack of effect in the current study. It
420 might also be the case that this subject population, although metabolically at-risk, were not sufficiently
421 compromised by increased sympathetic activity at baseline in order to induce a measurable increase in HRV.
422 The effect of central adiposity on insulin resistance is thought to be mediated partly via chronic low-
423 grade inflammation [65]. Therefore, IER may confer a greater increase in insulin sensitivity relative to CER
424 [29,30] by acutely modulating production of systemic pro-inflammatory cytokines (IL-6, IL-8, TNF-α, IL-
425 1β, MCP-1), the IL-1 receptor antagonist, IL-1Ra, all of which have been shown to be correlated with insulin
426 resistance [66–70], and the angiogenic growth factor VEGF which is associated with visceral fat and other
427 components of the metabolic syndrome [71]. The lack of effect of either diet on a summary score of low-
428 grade inflammation, despite weight loss and reductions in waist circumference (indicating reductions in
429 intra-abdominal adiposity), may be related to the basal low burden of inflammation in this mainly healthy
430 population. One study previously reported decreased TNF-α in overweight adults with asthma following 8
431 weeks of alternate day fasting, although there was no control group so this change cannot be attributed to the
432 pattern of energy restriction [72]. Nevertheless, overweight adults with asthma are likely to have a greater
433 burden of chronic low-grade inflammation compared to overweight adults with no overt medical conditions,
434 as indicated by the lack of effect on IL-6 and TNF-α in healthy populations with obesity following alternate
435 day fasting (comparing before and after) [73], or on IL-6, TNF-α and CRP following a 5:2 pattern fasting
436 relative to CER [29,30].
437 Despite the higher fasting glucose concentrations following IER relative to CER, a difference which,
438 although statistically significant, is unlikely to be clinically significant, the findings from this study generally
439 support the use of IER in the short-term for weight loss and cardiometabolic benefit as an equally effective
440 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

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

Conflict of interest

The authors have nothing to disclose.

Acknowledgements

The authors thank the study participants for taking part in the study. The authors also thank Dr Haya Al-Khatib for venepuncture services.

Funding sources

This work was supported by Lighterlife (UK) Ltd, who funded the running costs of the trial and provided the foodpacks used for the IER fasting days.

Authors' contributions to the manuscript

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

496 **References**

497 [1] Pi-Sunyer X. The Medical Risks of Obesity. Postgrad Med 2009;121:21–33.

498 [2] Jung U, Choi M-S. Obesity and Its Metabolic Complications: The Role of Adipokines and the
499 Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic
500 Fatty Liver Disease. Int J Mol Sci 2014;15:6184–223. doi:10.3390/ijms15046184.

501 [3] Klein S, Allison D, Heymsfield S, Kelley D, Leibel R, Nonas C, et al. Waist circumference and
502 cardiometabolic risk: a consensus statement from Shaping America's Health: Association for Weight
503 Management and Obesity Prevention. Obesity 2007;15:1061–7. doi:10.2337/dc07-9921.

504 [4] Jensen MD. Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model.
505 Obesity 2006;14:20S-24S. doi:10.1038/oby.2006.278.

506 [5] Chaston TB, Dixon JB, O'Brien PE. Changes in fat-free mass during significant weight loss: A
507 systematic review. Int J Obes 2007;31:743–50. doi:10.1038/sj.ijo.0803483.

508 [6] Browning JD, Baker JA, Rogers T, Davis J, Satapati S, Burgess SC. Short-term weight loss and
509 hepatic triglyceride reduction: Evidence of a metabolic advantage with dietary carbohydrate
510 restriction. Am J Clin Nutr 2011;93:1048–52. doi:10.3945/ajcn.110.007674.

511 [7] Maxwell MH, Kushiro T, Dornfeld LP, Tuck ML, Waks AU. BP Changes in Obese Hypertensive
512 Subjects During Rapid Weight Loss: Comparison of Restricted v Unchanged Salt Intake. Arch Intern
513 Med 1984;144:1581–4. doi:10.1001/archinte.1984.00350200073012.

514 [8] Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, et al. Effect of Long-Term
515 Treatment with Metformin Added to Hypocaloric Diet on Body Composition, Fat Distribution, and
516 Androgen and Insulin Levels in Abdominally Obese Women with and without the Polycystic Ovary
517 Syndrome. J Clin Endocrinol Metab 2000;85:2767–74. doi:10.1210/jcem.85.8.6738.

518 [9] Lobley GE, Holtrop G, Bremner DM, Calder AG, Milne E, Johnstone AM. Impact of short term
519 consumption of diets high in either non-starch polysaccharides or resistant starch in comparison with
520 moderate weight loss on indices of insulin sensitivity in subjects with metabolic syndrome. Nutrients
521 2013;5:2144–72. doi:10.3390/nu5062144.

522 [10] Mouridsen MR, Bendsen NT, Astrup A, Haugaard SB, Binici Z, Sajadieh A. Modest weight loss in
523 moderately overweight postmenopausal women improves heart rate variability. Eur J Prev Cardiol
524 2013;20:671–7.

[11] Alvarez GE, Beske SD, Ballard TP, Davy KP. Sympathetic neural activation in visceral obesity. Circulation 2002;106:2533–6.

[12] Lindmark S, Lönn L, Wiklund U, Tufvesson M, Olsson T, Eriksson JW. Dysregulation of the autonomic nervous system can be a link between visceral adiposity and insulin resistance. Obes Res 2005;13:717–28.

[13] do Carmo JM, da Silva AA, Wang Z, Fang T, Aberdein, Nicola, de Lara Rodriguez CE. Obesity-Induced Hypertension: Brain Signaling Pathways. Curr Hypertens Rep 2016;18:58.

[14] Straznicky NE, Eikelis N, Lambert EA, Esler MD. Mediators of sympathetic activation in metabolic syndrome obesity. Curr Hypertens Rep 2008;10:440–7.

[15] Skrapari I, Tentolouris N, Perrea D, Bakoyiannis C, Papazafiropoulou A, Katsilambros N. Baroreflex sensitivity in obesity: relationship with cardiac autonomic nervous system activity. Obesity 2007;15:1685–93.

[16] Grassi G, Dell'Oro R, Facchini A, Quarti-trevano F, Bolla GB, Mancia G. Effect of central and peripheral body fat distribution on sympathetic and baroreflex function in obese normotensives. J Hypertens 2004;22:2363–9.

[17] Gamboa A, Okamoto LE, Arnold AC, Figueroa A, Diedrich A, Raj SR, et al. Autonomic Blockade Improves Insulin Sensitivity in Obese Subjects. Hypertension 2014;64:867–74.

[18] Raynor HA, Champagne CM. Position of the Academy of Nutrition and Dietetics: Interventions for the Treatment of Overweight and Obesity in Adults. J Acad Nutr Diet 2016;116:129–47. doi:10.1016/j.jand.2015.10.031.

[19] Hankey CR. Session 3 (Joint with the British Dietetic Association): Management of obesity - Weight-loss interventions in the treatment of obesity. Proc Nutr Soc 2010;69:34–8. doi:10.1017/S0029665109991844.

[20] Klempel MC, Kroeger CM, Varady KA. Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet. Metabolism 2013;62:137–43. doi:10.1016/j.metabol.2012.07.002.

[21] Harris L, McGarty A, Hutchison L, Ells L, Hankey C. Short-term intermittent energy restriction interventions for weight management: a systematic review and meta-analysis. Obes Rev 2018;19:1–13. doi:10.1111/obr.12593.

554 [22] Davis CS, Clarke RE, Coulter SN, Rounsefell KN, Walker RE, Rauch CE, et al. Intermittent energy
555 restriction and weight loss: A systematic review. Eur J Clin Nutr 2016;70:292–9.
556 doi:10.1038/ejcn.2015.195.
557 [23] Headland M, Clifton PM, Carter S, Keogh JB. Weight-loss outcomes: A systematic review and meta-
558 analysis of intermittent energy restriction trials lasting a minimum of 6 months. Nutrients 2016;8.
559 doi:10.3390/nu8060354.
560 [24] Cioffi I, Evangelista A, Ponzo V, Ciccone G, Soldati L, Santarpia L, et al. Intermittent versus
561 continuous energy restriction on weight loss and cardiometabolic outcomes : a systematic review and
562 meta - analysis of randomized controlled trials. J Transl Med 2018;16:371. doi:10.1186/s12967-018-
563 1748-4.
564 [25] Sundfør TM, Svendsen M, Tonstad S. Effect of intermittent versus continuous energy restriction on
565 weight loss, maintenance and cardiometabolic risk: A randomized 1-year trial. Nutr Metab
566 Cardiovasc Dis 2018;28:698–706. doi:10.1016/j.numecd.2018.03.009.
567 [26] Patterson RE, Sears DD. Metabolic Effects of Intermittent Fasting. Annu Rev Nutr 2017;21:371–93.
568 doi:10.1146/annurev-nutr-071816.
569 [27] Antoni R, Johnston KL, Collins AL, Robertson MD. Effects of intermittent fasting on glucose and
570 lipid metabolism. Proc Nutr Soc 2017;76:361–8. doi:10.1017/S0029665116002986.
571 [28] Seimon R V., Roekenes JA, Zibellini J, Zhu B, Gibson AA, Hills AP, et al. Do intermittent diets
572 provide physiological benefits over continuous diets for weight loss? A systematic review of clinical
573 trials. Mol Cell Endocrinol 2015;418:153–72. doi:10.1016/j.mce.2015.09.014.
574 [29] Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Cuzick J, et al. The effects of
575 intermittent or continuous restriction on weight loss and metabolic disease risk markers: a randomised
576 trial in young overweight women. Int J Obes 2011;35:714–27.
577 [30] Harvie M, Wright C, Pegington M, McMullan D, Mitchell E, Martin B, et al. The effect of
578 intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and
579 metabolic disease risk markers in overweight women. Br J Nutr 2013;110:1534–47.
580 [31] Hara T, Kimura I, Inoue D, Ichimura A, Hirasawa A. Free fatty acid receptors and their role in
581 regulation of energy metabolism. Rev Physiol Biochem Pharmacol 2013;164:77–116.
582 [32] World Health Organization. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert

583 Consultation 2008.
584 [33] A Misra, P Chowbey, BM Makkar, NK Vikram, JS Wasir, D Chadha, Shashank R Joshi, S Sadikot, R
585 Gupta, Seema Gulati YM for CG. Consensus Statement for Diagnosis of Obesity, Abdominal Obesity
586 and the Metabolic Syndrome for Asian Indians and Recommendations for Physical Activity, Medical
587 and Surgical Management. J Assoc Physicians India 2009;57:163–70.
588 [34] van Strien T, Frijters JER, Bergers GPA, Defares PB. The Dutch Eating Behavior Questionnaire
589 (DEBQ) for Assessment of Restrained , Emotional , and External Eating Behavior. Int J Eat Disord
590 1986;5:295–315. doi:10.1002/erv.2448.
591 [35] Carver CS, Scheier MF. Assessing Coping Strategies: A Theoretically Based Approach. J Pers Soc
592 Psychol 1989;56:267–83. doi:10.1037/0022-3514.56.2.267.
593 [36] M. SS, Stevenson R, Wu C, Rutledge S, Stark CE. Stability of age-related deficits in the mnemonic
594 similarity task across task variations. Behav Neurosci 2015;129:257–68.
595 [37] Fillion L, Puntillo K a, Viens C, Fortier M, City Q. Validation of the COSMED FitMate for
596 prediction of maximal oxygen consumption 2006;15:18–20.
597 [38] Nieman DC, Austin MD, Benezra L, Pearce S, McInnis T, Unick J, et al. Validation of Cosmed's
598 FitMate in measuring oxygen consumption and estimating resting metabolic rate. Res Sports Med
599 2006;14:89–96. doi:10.1080/15438620600651512.
600 [39] Gerrior S, Juan W, Basiotis P. An easy approach to calculating estimated energy requirements. Prev
601 Chronic Dis 2006;3:A129.
602 [40] Bauman A, Bull F, Chey T, Craig CL, Ainsworth BE, Sallis JF, et al. The international prevalence
603 study on physical activity: Results from 20 countries. Int J Behav Nutr Phys Act 2009;6:1–11.
604 doi:10.1186/1479-5868-6-21.
605 [41] Lowe MR, Butryn ML, Didie ER, Annunziato RA, Thomas JG, Crerand CE, et al. The Power of
606 Food Scale. A new measure of the psychological influence of the food environment. Appetite
607 2009;53:114–8. doi:10.1016/j.appet.2009.05.016.
608 [42] Perseghin G, Caumo A, Caloni M, Testolin G, Luzi L. Incorporation of the fasting plasma FFA
609 concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. J
610 Clin Endocrinol Metab 2001;86:4776–81. doi:10.1210/jc.86.10.4776.
611 [43] Wlazlo N, Van Greevenbroek MMJ, Ferreira I, Jansen EJHM, Feskens EJM, Van Der Kallen CJH, et

al. Low-grade inflammation and insulin resistance independently explain substantial parts of the association between body fat and serum C3: The CODAM study. Metabolism 2012;61:1787–96. doi:10.1016/j.metabol.2012.05.015.

[44] van Bussel BCT, Henry RMA, Schalkwijk CG, Dekker JM, Nijpels G, Stehouwer CDA. Low-grade inflammation, but not endothelial dysfunction, is associated with greater carotid stiffness in the elderly. J Hypertens 2012;30:744–52. doi:10.1097/HJH.0b013e328350a487.

[45] van Greevenbroek MMJ, Jacobs M, van der Kallen CJH, Vermeulen VMMJ, Jansen EHJM, Schalkwijk CG, et al. The cross-sectional association between insulin resistance and circulating complement C3 is partly explained by plasma alanine aminotransferase, independent of central obesity and general inflammation (the CODAM study). Eur J Clin Invest 2011;41:372–9. doi:10.1111/j.1365-2362.2010.02418.x.

[46] Boutcher YN, Boutcher SH. Cardiovascular response to Stroop: effect of verbal response and task difficulty. Biol Psychol 2006;73:235–41.

[47] Malik M, Bigger JT, Camm AJ, Kleiger RE, Malliani A, Moss AJ, et al. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 1996;17:354–81.

[48] Rabasa-Lhoret R, Bastard JP, Jan V, Ducluzeau PH, Andreelli F, Guebre F, et al. Modified Quantitative Insulin Sensitivity Check Index Is Better Correlated to Hyperinsulinemic Glucose Clamp than Other Fasting-Based Index of Insulin Sensitivity in Different Insulin-Resistant States. J Clin Endocrinol Metab 2003;88:4917–23. doi:10.1210/jc.2002-030316.

[49] Reidlinger DP, Darzi J, Hall WL, Seed PT, Chowienczyk PJ, Sanders TAB, et al. How effective are current dietary guidelines for cardiovascular disease prevention in healthy middle-aged and older men and women? A randomized controlled trial. Am J Clin Nutr 2015;101. doi:10.3945/ajcn.114.097352.

[50] Antoni R, Johnston KL, Collins AL, Robertson MD. Intermittent v. continuous energy restriction: differential effects on postprandial glucose and lipid metabolism following matched weight loss in overweight/obese participants. Br J Nutr 2018;119:507–16. doi:10.1017/S0007114517003890.

[51] Williams K V, Mullen ML, Kelley DE, Wing RR. The effect of short periods of caloric restriction on weight loss and glycemic control in type 2 diabetes. Diabetes Care 1998;21:2–8.

641 doi:10.2337/diacare.21.1.2.
642 [52] Conley M, Le Fevre L, Haywood C, Proietto J. Is two days of intermittent energy restriction per week
643 a feasible weight loss approach in obese males? A randomised pilot study. Nutr Diet 2018;75:65–72.
644 doi:10.1111/1747-0080.12372.
645 [53] Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, Bergman RN. Caloric Restriction Per Se Is
646 a Significant Factor in Improvements in Glycemic During Weight Loss in Obese. Diabetes Care
647 1994;17:30–6. doi:10.2337/diacare.17.1.30.
648 [54] Hill J, Schlundt D, Sbrocco T, Sharp T, Pope-Cordle J, Stetson B, et al. Evaluation of an alternating-
649 calorie diet with and without exercise in the treatment of obesity. Am J Clin Nutr 1989;50:248–54.
650 [55] Corpeleijn E, Saris WHM, Blaak EE. Metabolic flexibility in the development of insulin resistance
651 and type 2 diabetes: Effects of lifestyle: Etiology and Pathophysiology. Obes Rev 2009;10:178–93.
652 doi:10.1111/j.1467-789X.2008.00544.x.
653 [56] Meijssen S, Castro Cabezas M, Ballieux CGM, Derksen RJ, Bilecen S, Erkelens DW. Insulin
654 mediated inhibition of hormone sensitive lipase activity in vivo in relation to endogenous
655 catecholamines in healthy subjects. J Clin Endocrinol Metab 2001;86:4193–7.
656 doi:10.1210/jcem.86.9.7794.
657 [57] Soeters MR, Lammers NM, Dubbelhuis PF, Ackermans M, Jonkers-Schuitema CF, Fliers E, et al.
658 Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism. Am J Clin Nutr
659 2009;90:1244–51. doi:10.3945/ajcn.2008.27327.
660 [58] López-Jaramillo P, Gómez-Arbeláez D, López-López J, López-López C, Martínez-Ortega J, Gómez-
661 Rodríguez A, et al. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Horm
662 Mol Biol Clin Investig 2014;18. doi:10.1515/hmbci-2013-0053.
663 [59] Wronska A, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue
664 depots. Acta Physiol 2012;205:194–208. doi:10.1111/j.1748-1716.2012.02409.x.
665 [60] Klempel MC, Kroeger CM, Bhutani S, Trepanowski JF, Varady K a. Intermittent fasting combined
666 with calorie restriction is effective for weight loss and cardio-protection in obese women. Nutr J
667 2012;11:98.
668 [61] Klempel MC, Varady KA. Reliability of leptin, but not adiponectin, as a biomarker for diet-induced
669 weight loss in humans. Nutr Rev 2011;69:145–54. doi:10.1111/j.1753-4887.2011.00373.x.

[62] Do Carmo JM, Da Silva AA, Dubinion J, Sessums PO, Ebaady SH, Wang Z, et al. Control of metabolic and cardiovascular function by the leptin-brain melanocortin pathway. IUBMB Life 2013;65:692–8. doi:10.1002/iub.1187.

[63] Trepanowski JF, Kroeger CM, Barnosky A, Klempel MC, Bhutani S, Hoddy KK, et al. Effect of alternate-day fasting on weight loss, weight maintenance, and cardioprotection among metabolically healthy obese adults: A randomized clinical trial. JAMA Intern Med 2017;177:930–8. doi:10.1001/jamainternmed.2017.0936.

[64] Mager DE, Wan R, Brown M, Cheng A, Wareski P, Abernethy DR, et al. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. FASEB J 2006;20:631–7.

[65] Shoelson SE, Lee J, Goldfine AB. Review series Inflammation and insulin resistance. J Clin Invest 2006;116:1793–801. doi:10.1172/JCI29069.and.

[66] Phillips CM, Perry IJ. Does Inflammation Determine Metabolic Health Status in Obese and Nonobese Adults? J Clin Endocrinol Metab 2013;98:E1610–9. doi:10.1210/jc.2013-2038.

[67] Kim C-S, Park H-S, Kawada T, Kim J-H, Lim D, Hubbard NE, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes (Lond) 2006;30:1347–55. doi:10.1038/sj.ijo.0803259.

[68] Vandanmagsar B, Youm Y-H, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011;17:179–88. doi:10.1038/nm.2279.

[69] Esser N, L'homme L, De Roover A, Kohnen L, Scheen AJ, Moutschen M, et al. Obesity phenotype is related to NLRP3 inflammasome activity and immunological profile of visceral adipose tissue. Diabetologia 2013;56:2487–97. doi:10.1007/s00125-013-3023-9.

[70] Ballak DB, Stienstra R, Tack CJ, Dinarello CA, van Diepen JA. IL-1 family members in the pathogenesis and treatment of metabolic disease: Focus on adipose tissue inflammation and insulin resistance. Cytokine 2015;75:280–90. doi:10.1016/j.cyto.2015.05.005.

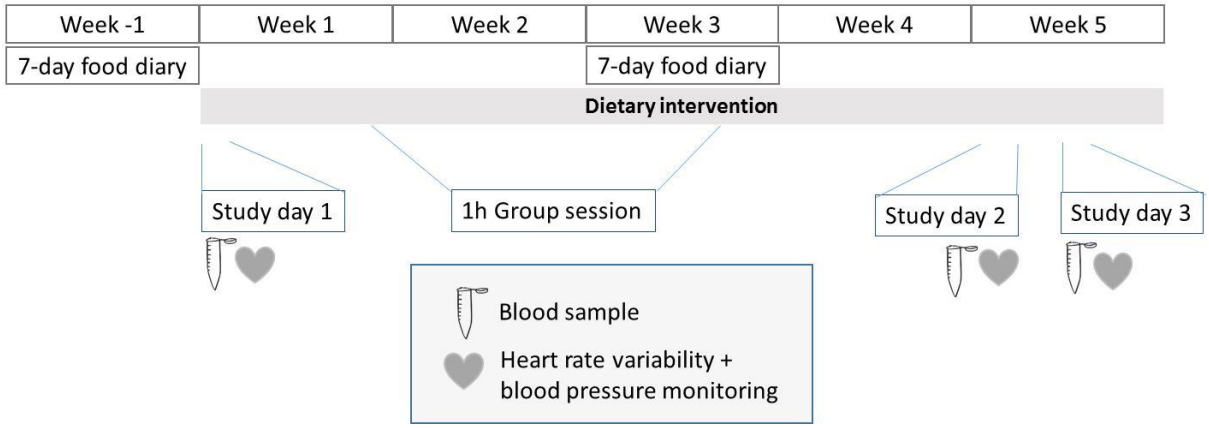
[71] Mazidi M, Rezaie P, Kengne AP, Stathopoulou MG, Azimi-Nezhad M, Siest S. VEGF, the underlying factor for metabolic syndrome; fact or fiction? Diabetes Metab Syndr Clin Res Rev 2017;11:S61–4. doi:10.1016/j.dsx.2016.12.004.

[72] Johnson JB, Summer W, Cutler RG, Martin B, Hyun D-H, Dixit VD, et al. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radic Biol Med 2007;42:665–74. doi:10.1016/j.freeradbiomed.2006.12.005.

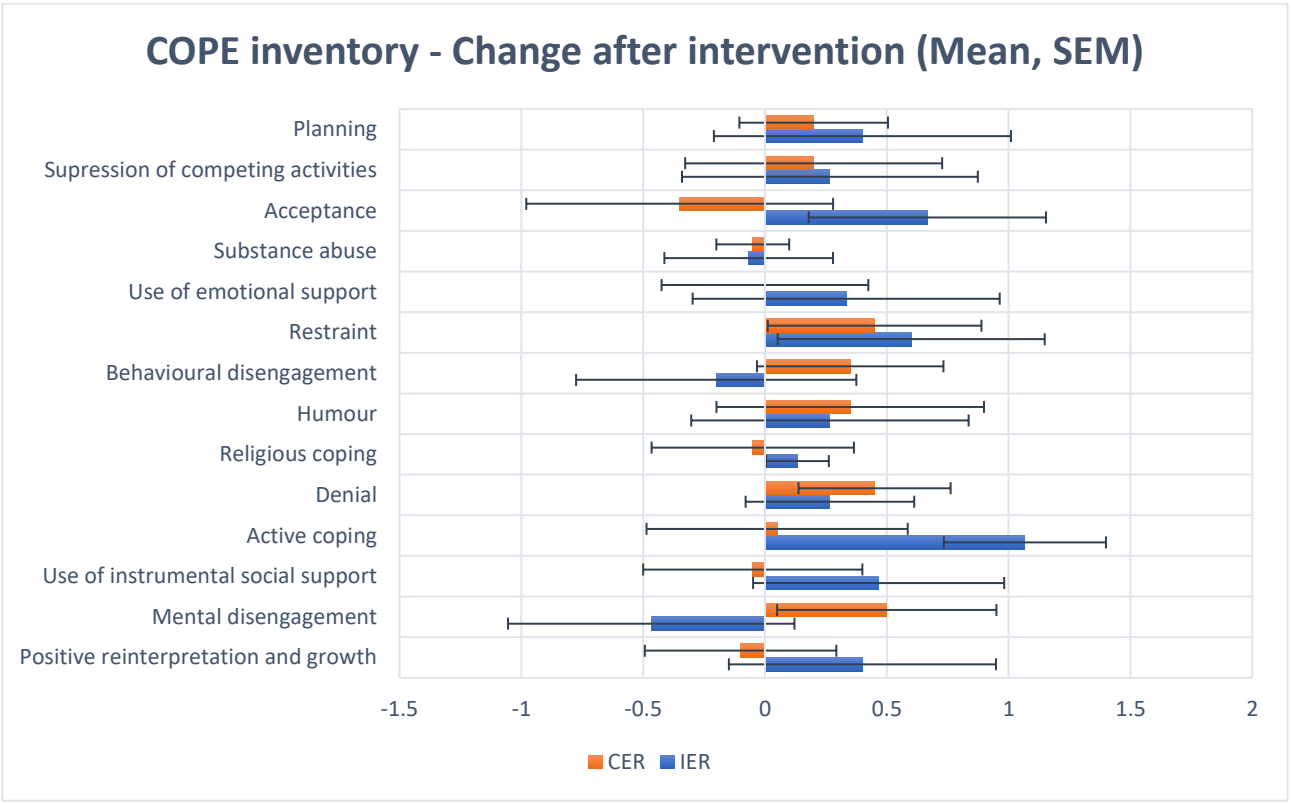
[73] Halberg N, Henriksen M, Söderhamn N, Stallknecht B, Ploug T, Schjerling P, et al. Effect of intermittent fasting and refeeding on insulin action in healthy men. J Appl Physiol 2005;99:2128. doi:10.1152/japplphysiol.00683.2005.

[74] Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013;31:1281–357. doi:10.1093/eurheartj/eht151.

Supplementary Figure 1. Study timeline.



Supplementary Figure 2. COPE inventory.



Supplementary table 1. Cardiometabolic effects of the intervention on the whole cohort.

	Whole cohort		
	Baseline	**END**	***P* 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/m^2)	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

END, measurements 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; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; IBI, interbeat interval; bpm, beats per minute; SDNN, standard deviation of all 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. Comparison within whole cohort by paired T-test or Wilcoxon signed rank test for related samples. Statistically significant values are in bold. [a] Geometric means with 95% CI; [b] Median (lower and upper IQR); [c] *p* value obtained using related samples Wilcoxon Signed Rank Test; [d] 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 score($_{LN}$MCP).

Supplementary table 2. Inflammatory markers

	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**	***p* value [1]**	**Baseline [c]**	**END [c]**	***p* value [2]**	**2-d SER[d]**	***p* value [2]**	**Mean differences[3]**	***p* 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

CER, continuous energy restriction; IER, intermittent energy restriction; IL, interleukin; 2-d SER, measurements taken following 2-d severe energy restriction; END, 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 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 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] 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 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 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 following LN transformation; there were no differences between groups.