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The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomised trial in young overweight women

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Abstract

Background—The problems of adherence to energy restriction in humans are well known.

Objective—To compare the feasibility and effectiveness of IER with CER for weight loss, insulin sensitivity and other metabolic disease risk markers.

Design—Randomised comparison of a 25% energy restriction as IER (~2266 kJ/day for 2 days/week) or CER (~6276 kJ/day for 7 days/week) in 107 overweight or obese (mean [±SD] body mass index 30.6 [±5.1] kg/m^2) premenopausal women over 6 months. Weight, anthropometry, biomarkers for breast cancer, diabetes, cardiovascular disease and dementia risk; insulin resistance (HOMA), oxidative stress markers, leptin, adiponectin, IGF-1 and IGF binding proteins 1 and 2, androgens, prolactin, inflammatory markers (high sensitivity C-reactive protein and sialic acid), lipids, blood pressure and brain derived neurotrophic factor were assessed at baseline and after 1, 3 and 6 months.

Results—Last observation carried forward analysis showed IER and CER are equally effective for weight loss, mean (95% confidence interval [CI]) weight change for IER was −6.4 (−7.9 to −4.8) kg vs.−5.6 (−6.9 to −4.4) kg for CER (P value for difference between groups = 0.4). Both groups experienced comparable reductions in leptin, free androgen index, high sensitivity C-reactive protein, total and LDL cholesterol, triglycerides, blood pressure and increases in sex hormone binding globulin, IGF binding proteins 1 and 2. Reductions in fasting insulin and insulin resistance were modest in both groups, but greater with IER than CER; difference between groups for fasting insulin −1.2 [−1.4 to −1.0] μU/ml, and insulin resistance −1.2 [−1.5 to −1.0] μU/mmol/L (both P=0.04).

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None of the authors have any conflicts of interest.

Conclusion—IER is as effective as CER in regards to weight loss, insulin sensitivity and other health biomarkers and may be offered as an alternative equivalent to CER for weight loss and reducing disease risk.

Keywords

intermittent; continuous energy restriction; randomised; premenopausal women; insulin sensitivity

Introduction

Excess weight and weight gain during adult life increases the risk of several diseases including diabetes (1), cardiovascular disease (CVD) (2), dementia (3), certain forms of cancer including breast cancer (4), and can contribute to premature death (5). Observational and some randomised trials indicate that modest weight reduction (>5% of body weight) reduces the incidence (6) (7) and progression (8) of many of these diseases. Although weight control is beneficial, the problem of poor compliance in weight loss programmes is well known (9). Even where reduced weights are maintained, many of the benefits achieved during weight loss, including improvements in insulin sensitivity, may be attenuated due to non-compliance or adaptation (10). Sustainable and effective energy restriction strategies are thus required. One possible approach may be intermittent energy restriction (IER), with short spells of severe restriction between longer periods of habitual energy intake. For some subjects such an approach may be easier to follow than a daily or continuous energy restriction (CER) and may overcome adaption to the weight reduced state by repeated rapid improvements in metabolic control with each spell of energy restriction (11).

The effect of IER on disease prevention and lifespan has been studied mainly in rodent models using a range of experimental protocols from every other day fasting to 3 weeks of partial energy restriction and refeeding. In these studies IER appears equally or more effective than isoenergetic CER for improving insulin sensitivity (12) preventing spontaneous or genetically engineered mammary tumours (13) (14), delaying the onset of prostate cancer (15), increasing resistance to neuronal damage (12), reducing cognitive impairment (16), protecting the heart (17) and increasing lifespan of rodents (18). IER may even produce similar benefits to those observed following more stringent CER (14). Few human studies have examined the effects of IER, possibly due to concerns of disordered eating patterns and over -consumption on non-restricted days. Several short term studies suggest that this does not occur (19;20). We report a randomised trial of 25% energy restriction delivered as IER versus CER in overweight or obese premenopausal women over a 6 month period, exploring the relative effects of the two dietary approaches on anthropomorphic and metabolic variables.

Subjects and methods

Subjects

We studied 107 premenopausal women aged 30 to 45 years with adult weight gain since the age of 20 exceeding 10kg, and a body mass index (BMI) between 24 and 40 kg/m^2. We recruited women from our Breast Cancer Family History Clinic, and women from the general population. As such, 54% of recruits had a family history of breast cancer (lifetime risk >1 in 6) (Tyrer Cuzick model) (21). Participants were non-smokers, not currently dieting or losing weight, with regular menstrual cycles and no evidence of hyperandrogenism or polycystic ovary syndrome (22), and no oral contraceptive use during the previous 6 months. They did not have high intakes of alcohol (>28 units/week) or phytoestrogens, and were not suffering from diagnosed diabetes, CVD, major psychiatric morbidity or cancer. We solicited participants from our Family History Clinic by mail shot,

and women in the general population using the media and institution wide e-mails. Potential participants were screened by the study dietitians (MH, MP) to assess their physical and psychological health and motivation to lose weight, and successfully completed a 2 day trial of the very low calorie diet (VLCD) diet prior to recruitment. Of 135 who were eligible after screening, 13 (9%) did not believe they could tolerate the diet for the 6 month trial period, a further 14 (10%) decided not to participate due to social, health or work related factors (Figure 1). All participants gave informed consent. The protocol was approved by the South Manchester Ethics Committee (reference 05/Q1403/243).

Study protocol

Participants were stratified according to BMI (above or below the predicted median value 28 kg/m^2), family history of breast cancer, sedentary (< or >1 hour moderate activity/week), and also according to the evaluating study dietitian to ensure the 2 dietitians saw equal proportions of patients from the two treatment groups. Women were randomly assigned to 6 months of either the CER of 25% restriction below estimated requirements 7 days/week or the IER of 25% restriction delivered as a VLCD for 2 days/week with no restriction on the other 5 days/week.

Measurements were made before starting and at 1, 3 and 6 months. These included weight, total body fat, fat free mass (FFM) determined by impedance (Tanita TBF-300A, Tanita Europe BV, Middlesex UK) waist, hip, bust and thigh circumference, systolic and diastolic blood pressure (BP) (Omron M5-1 Omron Healthcare Limited, Milton Keynes UK) and blood sampling. All assessments were conducted in the morning after a 12 hour fast. Weight and body fat were assessed wearing light clothing. Body circumferences were measured in triplicate according to study protocols (23). BP was measured in triplicate after 10 minutes at rest and the mean value calculated. The IER group were assessed at least 5 days after their weekly 2 day VLCD to avoid any potential acute effects of the 2 day restriction on serum markers (11). Additional fasting serum samples were however collected in a subset of the IER group (n=15) after either 1 or 3 months of dietary intervention to ascertain acute effects of the diet on serum markers. Samples were collected after 5 days of normal intake (Monday) on the morning after the 2 day VLCD (Wednesday) and after 2 days of normal intake (Friday) and also on these days of the week in a subset of the CER group (n=9) for comparison.

Adherence to the dietary interventions at 1, 3 and 6 months was assessed using 7-day food diaries checked for completeness with the respondent. Mean energy, protein, fat and carbohydrate intakes were estimated using the Compeat 4 Nutrition Analysis System (Carlson Bengston Consultants, London UK). In addition the IER group were asked to record whether they had successfully completed either 2, 1 or 0 days VLCD each week during the study period. We estimated the proportion of the IER and CER groups adhering to the diets at each time point defined as the numbers of IER reporting 2 or 1 day VLCD each week and the number of CER achieving a 25% energy restriction. Physical activity was assessed using the validated international physical activity questionnaire (IPAQ) expressed as metabolic energy turnover (MET) minutes/day and kJ/day (24). Throughout the 6 month trial period participants were asked to report any adverse or positive physical or psychosocial effects of the interventions. Quality of life was assessed using the RAND SF-36 scale, reported as physical and mental component summary scores (25).

Participants were asked to record the first day of each menstrual cycle to ascertain any effects of the diets on menstrual cycle length. We did not attempt to time assessments in relation to the menstrual cycle but day of cycle was recorded and adjusted for in the analysis to account for variation in hormone and lipid biomarkers related to the cycle (26;27).

Experimental diets

Both diets involved a 25% energy restriction from estimated baseline energy requirements using reported METs x estimated basal metabolic rate (28).

The CER group were prescribed a daily 25% restriction based on a Mediterranean type diet (30% fat, 15% monounsaturated, 7% saturated fat, 7% polyunsaturated fatty acids, 45% low glycaemic load carbohydrate, and 25% protein) (29). The IER group were asked to undertake a VLCD (75% restriction) on 2 consecutive days and to consume estimated requirements for weight maintenance for the remaining 5 days according to the nutrient composition above. The VLCD provided 2060 to 2266 kJ of energy and 50 g protein/day and comprised 1.136 litres (2 pints) of semi skimmed milk, 4 portions of vegetables (~80 g/portion), 1 portion of fruit, a salty low calorie drink and a multivitamin and mineral supplement. Participants were advised to maintain their current activity levels throughout the trial, and did not receive specific exercise counselling. Energy prescriptions were reviewed throughout the trial to account for changes in weight and exercise levels to maintain a 25% restriction below estimated requirements for weight maintenance.

Diets were not provided to participants but were self selected using detailed individualised food portion lists, meal plans and recipes. To maximise compliance patients received fortnightly motivational phone calls and monthly clinic appointments where weight and anthropometrics were measured and reported back to patients. All subjects were encouraged to use cognitive behavioural techniques such as self monitoring, obtaining peer/family support and stimulus control to maintain diets (30).

Serum markers of disease risk

Fasting insulin, glucose, lipid levels and sex steroid hormones were measured at the Clinical Biochemistry Department at University Hospital of South Manchester NHS Foundation Trust with the following methods: insulin (electrochemoluminescence immunoassay, Elecsys Roche Diagnostics, Lewes England, within batch coefficient of variation [CV] 1.9%), glucose (hexokinase/glucose-6-phosphate inter-assay dehydrogenase method, Bayer Newbury England, CV 3%), sex hormone binding globulin (SHBG) (non-competitive IRMA, IRMA-Orion Diagnostica Oy Espoo Finland, CV 2.7%), prolactin (electrochemoluminescence immunoassay, Elecsys Roche Diagnostics, Lewes England, CV 0.8%). Androgens were assessed using liquid chromatography and tandem mass spectrometry (LC-MS/MS) with the following CVs: testosterone 6.9%, dehydroepiandrosterone sulphate (DHEAS) 7.3%, androstenedione 2.5%. Fasting insulin and glucose were combined to calculate the insulin resistance index using the homeostasis model assessment (HOMA) (31), whilst free androgen index (FAI) was also estimated by the equation 100 x serum testosterone/serum SHBG (32). Colorimetric enzyme reactions were used to measure total cholesterol (CV 0.8%), triglycerides (CV 1.5%) and HDL cholesterol (CV 1.0%) (all Roche Modular E170, Roche, UK). Levels were measured spectrophotometrically by an automated Olympus AU600 analyser. LDL cholesterol was calculated using the formula of Friedewald et al (33). The adipokines leptin and adiponectin and the inflammatory markers high sensitivity C-reactive protein (hsCRP) and sialic acid were determined at the MRC Human Nutrition Research Unit, Cambridge. Plasma leptin concentration was measured using an ELISA method (R&D Systems, Quantikine Human Leptin kit R&D Systems, Inc. Minneapolis, USA, CV 10%), whilst plasma adiponectin was measured using radioimmunoassay (LINCO Research Inc., Missouri USA, CV 10%). We also determined the ratio of leptin: adiponectin which has been linked to insulin sensitivity and breast cancer risk(34;35).

Sialic acid was assayed using a colorimetric assay (Roche, Welwyn Garden City UK, CV 1.2%) adapted for use on the Hitachi 912 Clinical Analyser (Roche) and hsCRP using a high-sensitivity particle enhanced turbidometric assay (Dade-Behring, Walton UK, CV 4.5%).

Total IGF-1 (CV 3.2%), ultra-filtered free IGF-1 (CV 12%), and binding proteins IGFBP-1 (CV 5.3%) and IGFBP-2 (CV 5.0%) were assayed at the Medical Research Laboratories, Aarhus University Hospital, Denmark as previously described (36) (37). Serum total ketone bodies (beta-hydroxybutyric acid (~80%) and acetoacetone) (CV 1.6%), brain derived neurotrophic factor (BDNF) (CV 2.9%) and ghrelin (CV 6.7%) were measured at the National Institute on Ageing (Baltimore, MD, USA) as previously described (38). Serum advanced oxidation protein products (AOPP) were measured using a modified method of Selmeci et al (CV 2.2%) (39). All serum and plasma samples were stored at 4 °C for no longer than 4 hours, aliquoted and frozen at −70 °C within 24 hours and batched so that all samples from a participant were included in the same assay (40). Laboratory personnel were blinded to the sample identity.

Statistical analysis

Data at baseline, 1, 3 and 6 months are presented as the mean (95% confidence intervals [CI]) or geometric mean (95% CI) for the log transformed variables (fasting insulin, insulin resistance, adiponectin, hsCRP, total IGF-1, IGFBP-1, IGFBP-2, ghrelin, total ketone bodies, fast and slow acting AOPP, androstenedione, DHEAS, SHBG, FAI, leptin, leptin: adiponectin ratio, and physical activity [MET min/day and kJ/day]).

The primary aim of the study was to determine changes in weight and insulin resistance between IER and CER over the 6 month weight loss period. Power calculations suggested an 80% power to detect a 25% difference in change in mean insulin resistance, allowing for a 15% drop out. The primary analysis was a last observation carried forward (LOCF) analysis of variance (ANOVA) at 6 months between the groups defined at randomisation adjusted for baseline levels of each parameter, day of menstrual cycle at assessment and change in physical activity over 6 months. A baseline observation carried forward analysis and a per protocol analysis of completers only showed comparable results to the LOCF.

We also assessed changes in weight, biomarkers, dietary intake and physical activity within each of the group using paired t tests at baseline and LOCF at 6 months. Statistical significance was accepted at $P<0.05$ for 6 month analysis and $P<0.01$ for other time points to adjust for multiple comparisons. Data were analysed using SPSS (version 14 SPSS Ltd, Chicago, IL, USA).

Changes in weight, body fat, waist and insulin resistance, over the trial period were also measured using generalised estimating equations (GEE) to allow all 3 time points to be analysed simultaneously, and to incorporate data from subjects with less than 3 time points without the need for substitution, thus increasing statistical power and a more efficient comparison across the various time points. These GEE models were constructed in Stata 10 (StataCorp LP, TX, USA) with an exchangeable correlation structure, the predictors used were the 3 time points (1, 3, 6 months), the group variable (IER vs. CER) and the group by time interaction.

Results

Baseline data

Characteristics of the groups at baseline are reported in Table 1. The groups were of comparable age, weight and demographics and were mainly Caucasian. A small number had

co-morbidities, which were equally frequent in the two groups. Six IER (11%) and 10 CER (18%) met the Diabetes Federation Criteria for the metabolic syndrome (41). The majority of subjects reported previous attempts to diet (IER 92%, CER 78%), with comparable previous attempts between the groups; IER 2.8 (2.1) and CER 2.4 (1.9) (P=0.29).

Eighteen women withdrew from the study before 6 months (IER=11, CER=7) representing 21% IER and 13% CER subjects (χ^2 =1.16, P=0.28). The main reasons for drop out were comparable between the groups: stress (IER=3, CER=2), pregnancy (IER=2, CER=1), change in employment (IER=2, CER=1), problems adhering to the diet (IER=3, CER=3) and personal illness (infected pacemaker, IER=1).

Changes in weight, body composition and circumferences

Weight loss was comparable between the groups. LOCF analysis at 6 months showed weight reduced from mean (95% CI) 81.5 (77.5 to 85.4) kg to 75 (71.2 to 78.8) kg in the IER group compared to a reduction from 84.4 (79.7 to 89.1) kg to 78.7 (74.2 to 83.2) kg in the CER group. The percentage of women in the IER and CER groups losing 5–10% body weight were 30 and 33% respectively, and losing 10% or more body weight were 34 and 22% respectively (χ^2=1.89, P=0.39). Both groups experienced comparable reductions in body fat, FFM, hip, bust and thigh circumference and composition of weight loss. Percentage of weight lost which was fat in the IER and CER groups was 79 (±24) and 79 (±26) % respectively (P=0.99) (Table 2). GEE modelling over 6 months showed no group or group by month interactions for weight (P=0.41) (Figure 2a) or body fat (Figure 2b) (P=0.36) but a non-significant greater decline in waist measurement with IER at three months (mean difference between groups [95% CI] −1.1 [−2.3 to 0.1] cm, group by month 3 interaction P=0.07) (Figure 2c).

Adherence

Weekly dietary records were available for 82 (76%) subjects at baseline, 72 (67%) at 1 month, 65 (60%) at 3 months and 58 (54%) at 6 months. There were no significant differences in energy or macronutrient intakes between the groups at baseline. Changes in dietary intake during the study are reported in Table 3. Both groups reported reductions in average weekly energy and macronutrient intakes, however the IER group reported greater reductions for average daily intake of energy (mean difference between groups [95% CI] −716 [−1240 to −192] kJ, −9 [−14 to −2] %, P<0.01), protein (−5.5 [−10.0 to −0.8] g, −6 [−13.0 to 0.0] %, P=0.02) and carbohydrate (−24 [−41 to −8] g, −11 [−18 to −3] %, P=0.004).

Intention to treat analysis assuming women who left the study or who did not complete food diaries did not adhere to the diets shows reported adherence to 2 days VLCD amongst the IER group to be 63% at 1 month, 43% at 3 months and 44% at 6 months. A further 7, 24, and 13% of IER subjects completed one day of VLCD at 1, 3 and 6 months respectively. The proportion of CER subjects reporting adhering to the 25% CER was 46% at 1 month, 37% at 3 months and 32% at 6 months. Completers only analysis showed adherence to 2 days or 1 day VLCD in the IER group to be respectively 70 and 8% at 1 month, 56 and 32% at 3 months and 64% and 19% at 6 months, whilst the 25% CER was achieved by 71% at 1 month, 61% at 3 months and 55% at 6 months. At the end of the trial 31 of IER (58%) and 46 (85%) of CER subjects planned to continue the diet allocated at randomisation. Neither group received counselling on exercise, there was no overall change in physical activity in either group.

Changes in insulin sensitivity and associated markers

Both groups experienced modest declines in fasting serum insulin and improvements in insulin sensitivity which were greater amongst the IER group (Table 4). Mean difference between groups [95% CI] for fasting insulin was −1.2 [−1.4 to −1.0] μU/ml, −16 [−19 to −13] %, P=0.04; and for insulin resistance was −1.2 [−1.5 to −1.0] μU/mmol/L, −45 [−86 to −3] %, P=0.04) (Table 4). GEE modelling showed that the IER group had greater reductions in insulin resistance than the CER group at 3 months (mean difference [95% CI] between groups −17 [−33.2 to −0.2] %, group by month 3 interaction, P=0.046) and 6 months (−23 [−38.1 to −8.6] %, group by month 6 interaction, P=0.001) (Figure 2d). Correspondingly there was a modest increase in adiponectin in the IER group but not the CER group, (mean difference [95% CI] +9 [−2 to 21] %, P=0.08). Changes in the IGF-axis were comparable between the groups with increased IGFBP-1 and IGFBP-2 but negligible changes in total and free IGF-1.

Both groups experienced modest decreases in the inflammatory marker hsCRP, but no change in sialic acid levels. The groups had comparable reductions in the oxidative stress marker, fast acting AOPP by 6 months, which appeared to occur earlier in IER compared to CER. Slow acting AOPP appeared to decrease in the IER group and have a slight increase in the CER group (mean difference between groups at 6 month [95% CI] −10 [−19 to 2] %, P=0.12). Women in the IER group had a non-significant greater increase in serum total ketone bodies at 6 months compared to the CER group suggesting higher rates of fat oxidation (mean difference between groups [95% CI] 33 [−8 to 93] %, P=0.12). There were no significant changes in either group for ghrelin, the growth factor BDNF or for fasting glucose.

Breast cancer risk markers

Both groups experienced large reductions in serum leptin, decreases in the ratio of leptin: adiponectin, no changes in serum levels of testosterone, androstenedione and prolactin. The CER group had a greater reduction in DHEAS compared to IER (mean difference [95% CI] CER vs. IER −6 [−14 to 1] %, P=0.08) however both groups experienced comparable increases in SHBG and a decrease in FAI (Table 5). Menstrual cycle data was available for 44 IER (83%) and 47 CER (87%). During the 6 month study period the mean (±SD) length of menstrual cycle was significantly longer in the IER group compared to the CER group (29.7 [±3.8] vs. 27.4 [±2.7] days, P=0.002).

Cardiovascular risk markers

Both diets led to comparable reductions in total and LDL cholesterol, triglycerides, systolic and diastolic BP. Neither group experienced changes in HDL levels (Table 5)

Effects of IER and CER on serum markers over one week

A sub-set of women (15 IER and 9 CER) provided fasting serum samples over 1 week during the study period. The IER group demonstrated acute reductions in fasting insulin (−23%), HOMA (−29%) and triglycerides (−18%), in the morning after the 2 day VLCD which normalised within 2 days of resuming normal diet. There were no significant changes in the CER group (Figure 3).

Quality of life

There were no major adverse effects of the diets. A small number of the IER group (4, 8%), but none of the CER group experienced minor adverse physical symptoms including lack of energy, headaches, feeling cold and constipation. Eight (15%) of the IER and none of the CER complained of hunger, whilst a further 3 (6%) of the IER and 7 (13%) of the CER

group reported increased energy and improved health. Eight (15%) of the IER and 4 (7%) of the CER group reported minor adverse psychological effects including lack of concentration, bad temper and preoccupation with food, whilst 17 (32%) of the IER and 25 (46%) of the CER group reported increased self confidence and positive mood. Predictably both groups acknowledged the limited food choice of the diets; 55% IER and 53% CER. More of the IER group reported problems fitting the diet into daily routine; 51% IER vs. 30% CER. RAND SF-36 quality of life scores were available for 96 patients at baseline (88%), 91 at 1 and 3 months (84%) and 75 at 6 months (69%). There was a modest increase in the physical component summary score in the IER but not the CER group (mean difference [95% CI] 2.1 [−0.1 to 4.3] units, 4 [0.0 to 8.0] %, P=0.06). In comparison there was a slightly greater increase in the mental component summary score in the CER compared to the IER group (2.8 [0.1 to 5.6] units, 5 [0.0 to 12.0] %, P=0.04).

Discussion

Main findings

This is the largest randomised comparison of an isocalorific intermittent vs. continuous energy restriction to date in free living humans. Both approaches achieved comparable weight loss and improvements in a number of risk markers for cancer, diabetes and cardiovascular disease, for example reductions in fasting insulin, insulin resistance, leptin, the leptin: adiponectin ratio, free androgen index, inflammatory markers, lipids, blood pressure, increases in SHBG, IGFBP-1 and 2. IER was no easier to adhere to than CER, however it may be offered as an equivalent alternative to CER for weight loss and reducing disease risk.

Comparison with other studies

There has only been limited research of IER in humans. Two small short term (12 week) randomised studies have reported the effects of IER vs. CER. Ash et al compared an IER (4180 kJ liquid VLCD 4 days/week, 3 days ad libitum) vs. CER (6000 to 7000 kJ/day) amongst 9 men with type 2 diabetes and showed no difference in terms of weight or fasting insulin (20). Hill et al (19) compared alternating weeks of 2508, 3762, 5016 or 7254 kJ/day as compared to constant restriction of 5016 kJ/day in 16 moderately obese women and reported greater reductions in cholesterol in the IER group compared to the CER group (−14 vs. −6%). A further study amongst patients with type 2 diabetes showed beneficial effects of periodic VLCD (either 1 day/week or 5 consecutive days every 5 weeks) in addition to and not instead of a normal daily restriction (6180 to 7416 kJ/day). Predictably additional periods of VLCD led to greater weight loss, however the 5 day VLCD period had a beneficial effect on long term glycaemic control which was independent of weight change (42), suggesting possible metabolic benefits of IER.

In our study both IER and CER led to modest reductions in fasting serum insulin and improvements in insulin sensitivity which appeared greater in the IER group even 5 days after the 2 day VLCD. These parameters were predictably improved further during the 2 day VLCD, most likely linked to acute decreased levels of insulin and increased insulin receptor affinity with energy restriction (43). The biological significance of these improvements in insulin sensitivity in our population who were not particularly insulin resistant (only 16% of our subjects met the Diabetes Federation Criteria for the metabolic syndrome) is not known. IER appeared to bring about a modest increase in adiponectin which has a pivotal role in insulin sensitivity and the development and progression of cancer, heart disease and diabetes (44). We did not however observe any acute effects of IER on adiponectin, which contrasts to the 37% increase on alternate fasting days, previously reported amongst healthy weight men (45).

Neither IER nor CER led to appreciable changes in total or free IGF-1. Animal studies have shown reductions in IGF-1 with CER but not consistently with IER (12) (46). BDNF is up-regulated in inflammatory conditions and in the metabolic syndrome. Levels did not change with either of our test diets. Earlier studies have linked weight loss to decreased serum levels amongst overweight asthmatics (38) but increased levels amongst healthy overweight subjects (47). In our study both CER and IER led to the anticipated increases in serum levels of ghrelin (48).

Reductions in circulating sex steroid levels may reduce risk of breast cancer. The declines in FAI seen in both groups have been reported previously in premenopausal women (49). The greater reduction in DHEAS with CER may be advantageous and translate to greater reductions in breast cancer risk in women (50), in contrast to men where higher levels of DHEAS are linked to longevity (51). Conversely the greater average cycle length amongst the IER women may reduce breast cancer risk and reflect increased follicular length due to perturbations of the neuroendocrine axis (52). Neither group experienced changes in prolactin. Reductions in prolactin have previously been reported with much larger weight loss (−15%) (53), thought to be due to enhanced dopamine 2 receptor activation. Reductions in the leptin: adiponectin ratio in both groups may be linked to improved insulin sensitivity (35)and reduced breast cancer risk(34).

Recent reviews speculate that IER may be associated with greater disease prevention than CER due to increased cellular stress resistance, in particular increased resistance to oxidative stress. This is thought to be mediated by ‘hormesis’ whereby the moderate stress of energy restriction increases the production of cytoprotective, restorative proteins, antioxidant enzymes and protein chaperones (54). Alternate day fasting has been linked to increased SIRT-1 gene expression in muscle (55), and to greater neuronal resistance to injury compared to CER in C57BL/6 mice (12). The tendency for greater improvements in oxidative stress markers in our IER than in the CER group may support these assertions. Declines in long term protein oxidation product aggregates suggest IER as a possible activator of catabolism and autophagy.

Both of our groups demonstrated good adherence and weight loss at 6 months (64% IER and 55% CER achieved >5% weight loss) which may reflect the motivation of the participants and ongoing monitoring and motivational calls. A number of the IER group experienced minor adverse physical and mental symptoms with the IER. Despite this 57% were still undertaking either 1 or 2 milk days at 6 months, which is comparable but no better than adherence to long term popular diets (9). A recent blinded trial of a 2 day VLCD (1311 kJ/day) reported no adverse effects on cognition, energy levels, sleep or mood (56), suggesting symptoms are expected with VLCD and therefore experienced and could potentially be overcome with appropriate counselling. Importantly IER did not lead to overeating on non-VLCD days. A similar lack of energy compensation has been reported after a 36 hour fast amongst healthy weight subjects (57).

Strengths of study

Previous reported weight loss and benefits of intermittent restriction have been reported from single arm studies (38;58). Our randomised trial allows the effects of IER to be directly compared with those of the standard CER approach and shows comparable benefits. Good retention to the study (83% at 6 months) and completeness of trial assessments means our LOCF analysis informs the relative acceptability and efficacy of the diets. We chose a pragmatic IER regimen which provided a 25% energy restriction and required a simple non-proprietary VLCD to be taken over 2 days/week. We believe this to be a more achievable than previously studied regimens of alternate days fasting or VLCD (38) (58;59). We tested the diets amongst overweight and obese free living individuals since this group is likely to

derive metabolic benefit from energy restriction. We studied premenopausal women only to avoid the potential effects of sex or menopausal status on metabolic biomarkers. The benefits of IER and CER in older women or men cannot be extrapolated, however earlier reports suggest acceptability of intermittent VLCD may be greater amongst men than in women (42) (60).

Study limitations

Though longer than previous studies we did not assess the effects of IER and CER beyond 6 months to investigate their relative effects for maintenance of weight loss. Fewer of the IER group (58%) planned to continue with the regimen beyond 6 months compared to the CER group (85%) suggesting difficulties with long term adherence to IER. Further studies are needed to address issues related to adherence.

We assessed the effects of the two diets on a comprehensive range of serum biomarkers of disease risk. This approach does not take into account any local changes in production of these factors which may be more relevant to disease risk (61). Nor does it consider different isoforms of the hormones such as high molecular weight adiponectin and aceylated ghrelin (which are specifically linked to insulin sensitivity) (62).

Implications and future studies

Insulin sensitivity was assessed using HOMA which is an accepted method amongst non-diabetics (31). Future trials should however compare the effects of IER to CER in a pre-diabetic population using more rigorous methods to study insulin and glucose metabolism, for example glucose clamp techniques. The overall effects of IER on glycaemic control, for example both during and after IER each week compared to CER could also be ascertained from measuring HBA1c and fructosamine. Such studies could also examine the relative impacts of IER and CER on visceral, hepatic, intramuscular fat stores, and fat cell size which could preferentially decrease during the weekly spells of acute negative energy balance with IER(63) (64).

Our data is suggestive that periods of severe restriction may have different effects which may be important in the long term for disease prevention. However IER was no easier to adhere to than CER particularly in the longer term. Predictably, ease of following the diets varied between individuals. IER can be offered as an alternative to CER for reducing obesity and obesity-related disorders in some individuals. Psychosocial studies are required to better understand behavioural factors which can promote or reduce compliance to IER and CER regimens.

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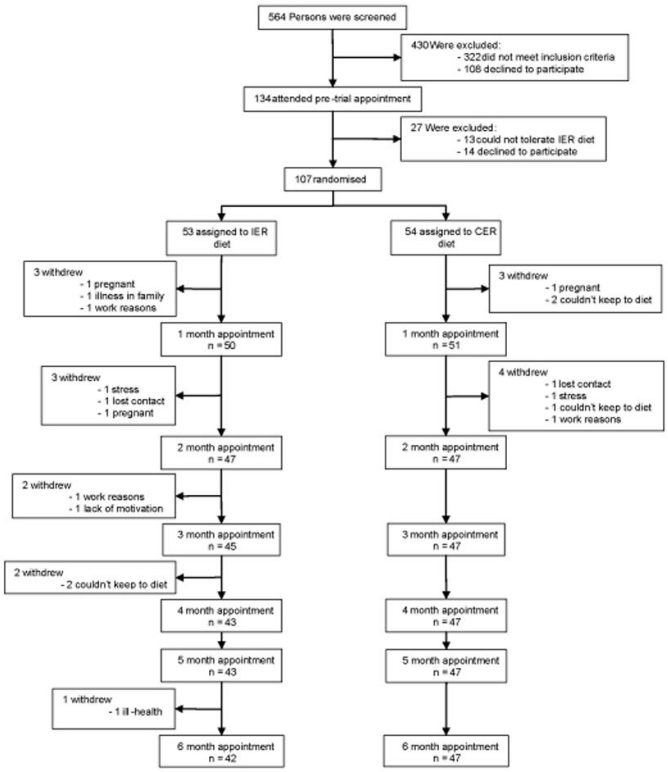

Figure 1.
Recruitment and retention to study

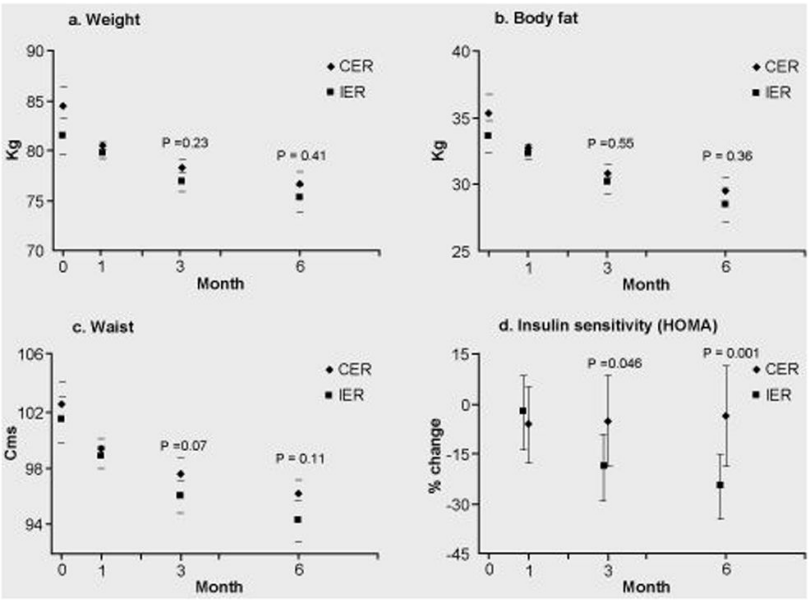

Figure 2.

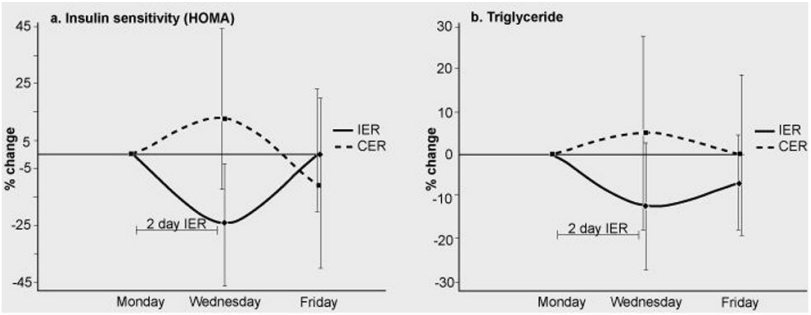

Figure 3.

Table 1

Baseline Characteristics of Subjects

	IER	CER	P Value
	N = 53	N = 54	
Age at start (years)[1]	40.1 (4.1)	40.0 (3.9)	0.85
Baseline BMI (kg/m^2)[1]	30.7 (5.0)	30.5 (5.2)	0.77
Weight gain since age 18 (kg)[1]	20.1 (11.0)	19.8 (10.5)	0.90
Family history of breast cancer (lifetime risk > 1 in 6)[2,3]	28 (54%)	30 (56%)	0.85
Sedentary <1 hour moderate activity/week[2]	23 (44%)	22 (41%)	0.70
Ethnic origin:[2]			0.21
Caucasian	50 (94%)	53 (98%)	
Afro Caribbean	1 (2%)	1 (2%)	
Other	2 (4%)	0 (0%)	
Married[2]	37 (69%)	39 (72%)	0.12
Children living at home[2]	52 (98%)	50 (92%)	0.55
Employment:[2]			0.32
Full-time	47 (88%)	41 (76%)	
Part-time	5 (9%)	10 (19%)	
Co morbidities:[2]			1.0
Asthma	5 (9%)	5 (9%)	
Hypertension	3 (6%)	2 (3%)	
Mild depression	0 (0%)	1 (2%)	
Medication:[2]			1.0
Anti-hypertensive	3 (6%)	4 (7%)	
Anti-inflammatories	2 (4%)	4 (7%)	
Steroid inhalers	5 (9%)	1 (2%)	
Thyroxin	1 (2%)	2 (4%)	
Anti-depressants	1 (2%)	1 (2%)	
Beta blockers	2 (4%)	1 (2%)	

[1] Mean (SD), Independent sample T test

[2] N (%), Chi Squared

[3] Tyrer-Cuzick model (21)

Table 2

Change in weight and circumferences over 6 months

Parameter		Baseline	1 Month	3 Month	6 Month	P value[2]
Weight (kg)	IER	81.5 (77.5 to 85.4)	79.7 (75.3 to 84.2)	77.4 (73.0 to 81.8)	75.8[1] (71.4 to 80.2)	0.26
	CER	84.4 (79.7 to 89.1)	83.4 (78.1 to 88.6)	81.4 (76.2 to 86.7)	79.9[1] (74.6 to 85.2)	
Body fat (kg)	IER	33.6 (30.9 to 36.4)	32.5 (29.3 to 35.7)	30.6 (27.5 to 33.8)	29.1[1] (26.0 to 32.3)	0.34
	CER	35.3 (31.9 to 38.7)	34.6 (30.8 to 38.3)	32.9 (29.1 to 36.6)	31.7[1] (27.9 to 35.5)	
Body fat %	IER	40.5 (39.0 to 42.0)	39.9 (38.0 to 41.7)	38.5 (36.5 to 40.5)	37.3[1] (35.2 to 39.3)	0.35
	CER	40.5 (38.7 to 42.3)	40.2 (38.2 to 42.2)	39.0 (36.9 to 41.1)	38.0[1] (35.8 to 40.3)	
Fat free mass (kg)	IER	47.6 (46.3 to 49.0)	46.9 (45.4 to 48.4)	46.5 (45.0 to 47.9)	46.4[1] (44.9 to 47.9)	0.21
	CER	49.1 (47.7 to 50.5)	48.8 (47.2 to 50.4)	48.5 (46.9 to 50.2)	48.3[1] (46.7 to 49.9)	
Waist (cm)	IER	101.5 (97.8 to 105.2)	99.5 (95.5 to 103.4)	97.3 (93.4 to 101.1)	95.4[1] (91.3 to 99.5)	0.13
	CER	102.5 (98.7 to 106.3)	101.3 (97.0 to 105.6)	99.8 (95.6 to 104.0)	98.6[1] (94.2 to 102.9)	
Hip (cm)	IER	111.0 (108.2 to 113.8)	109.3 (106.2 to 112.4)	107.3 (104.2 to 110.5)	106.2[1] (103.0 to 109.5)	0.23
	CER	111.6 (108.5 to 114.8)	111.0 (107.6 to 114.4)	109.2 (105.7 to 112.7)	108.2[1] (104.5 to 111.8)	
Bust (cm)	IER	105.3 (102.4 to 108.3)	103.9 (100.8 to 107.1)	102.0 (98.8 to 105.1)	100.5[1] (97.4 to 103.7)	0.19
	CER	105.5 (102.4 to 108.6)	103.9 (100.6 to 107.2)	102.4 (99.1 to 105.8)	101.2[1] (97.9 to 104.6)	
Thigh (cm)	IER	60.1 (58.2 to 62.0)	59.2 (57.3 to 61.1)	58.1 (56.1 to 60.0)	57.2[1] (55.2 to 59.1)	0.29
	CER	60.6 (58.5 to 62.8)	60.0 (57.8 to 62.2)	59.2 (57.0 to 61.5)	58.2[1] (56.0 to 60.4)	

Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months.

[1] Change from baseline to LOCF is statistically significant at 6 months within group $p<0.05$

[2] Analysis of variance (ANOVA) for LOCF at 6 months between groups adjusted for baseline levels of each parameter, change in physical activity over 6 months and day of menstrual cycle.

IER = intermittent energy restriction, CER = continuous energy restriction.

Baseline 53 IER and 54 CER, 1 month 51 IER and 51 CER, 3 months 45 IER and 47 CER, 6 months 42 IER and 47 CER.

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Table 3

Changes in dietary intake and physical activity over 6 months

Parameter		Baseline	1 Month	3 Month	6 Month	P value[4]
Energy (kcal/day)[1]	IER	1908.4 (1773.2 to 2043.5)	1348.6 (1254.8 to 1442.5)	1341.0 (1257.5 to 1424.6)	1340.9[3] (1243.9 to 1437.9)	0.01
	CER	1894.3 (1770.1 to 2018.4)	1425.5 (1315.0 to 1536.0)	1484.3 (1367.0 to 1601.7)	1506.8[3] (1390.9 to 1622.7)	
Energy (kJ/day)[1]	IER	7984.7 (7419.2 to 8550.1)	5642.7 (5249.9 to 6035.6)	5610.9 (5261.2 to 5960.5)	5610.4[3] (5204.5 to 6016.3)	0.01
	CER	7925.7 (7406.2 to 8445.2)	5964.3 (5502.0 to 6426.6)	6210.5 (5719.4 to 6701.5)	6304.5[3] (5819.6 to 6789.5)	
Protein (g/d)[1]	IER	80.3 (75 to 85.3)	73.2 (69.2 to 77.2)	72.1 (68.0 to 76.2)	70.7[3] (65.6 to 75.9)	0.02
	CER	77.3 (73.0 to 81.6)	71.9 (67.5 to 76.2)	74.6 (70.2 to 79.0)	73.4 (69.4 to 77.4)	
Fat (g/d)[1]	IER	73.0 (66.47 to 79.5)	43.3 (38.7 to 47.8)	43.7 (39.5 to 47.8)	43.7[3] (38.7 to 48.8)	0.11
	CER	73.2 (66.9 to 79.6)	48.1 (41.5 to 54.7)	51.6 (44.4 to 58.9)	50.4[3] (43.6 to 57.2)	
Saturated fat (g/d)[1]	IER	27.1 (24.0 to 30.2)	14.3 (12.3 to −9.5)	15.5 (13.7 to 8.7)	15.1[3] (13.1 to 8.7)	0.29
	CER	26.4 (23.8 to 29.1)	16.3 (13.8 to 18.8)	17.1 (14.2 to 20.0)	16.8[3] (14.1 to 19.5)	
Carbohydrates (g/d)[1]	IER	220.9 (202.0 to 239.7)	164.7 (154.3 to 175.1)	163.8 (153.3 to 174.2)	165.0[3] (153.5 to 176.5)	0.00
	CER	227.5 (212.6 to 242.4)	180.0 (167.1 to 192.9)	184.2 (171.1 to 197.3)	189.8[3] (174.5 to 205.0)	
Fibre (g/d)[1]	IER	13.6 (12.4 to 14.7)	13.2 (12.2 to 14.2)	12.8 (11.8 to 13.8)	13.1 (12.1 to 14.2)	0.00
	CER	13.9 (12.9 to 14.9)	14.9 (13.7 to 16.1)	14.9 (13.8 to 16.1)	15.9[3] 14.6 to 17.3)	
MET mins/day[2]	IER	178.1 (140.4 to 225.6)	245.3 (182.8 to 307.8)	236.7 (183.6 to 289.7)	243.5 (189.2 to 297.8)	0.98
	CER	218.0 (160.4 to 296.0)	300.0 (239.3 to 360.7)	326.2 (259.2 to 393.2)	373.9 (297.5 to 450.3)	
Energy expenditure for activity (kJ/day)[2]	IER	988.7 (776.3 to 1259.1)	1307.2 (948.5 to 1666.0)	1200.1 (922.3 to 1477.8)	1140.2 (880.6 to 1399.8)	0.75
	CER	1215.6 (845.1 to 1748.1)	1719.4 (1383.1 to 2055.7)	1865.0 (1439.4 to 2290.6)	2082.0 (1625.1 to 2538.8)	

[1]Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months.

[2]Geometric Mean (95% CI) for baseline values and LOCF values at 1, 3 and 6 months

[3]Change from baseline to LOCF at 6 months within group is statistically significant within group $p < 0.05$.

[4]Analysis of variance (ANOVA) for LOCF at 6 months between groups adjusted for baseline levels of each parameter

IER = intermittent energy restriction, CER = continuous energy restriction

Dietary intake data: Baseline 40 IER and 42 CER, 1 month 37 IER and 35 CER, 3 months 32 IER and 33 CER, 6 months 27 IER and 31 CER

Physical activity data: Baseline 50 IER and 52 CER, 1 month 49 IER and 47 CER, 3 months 42 IER and 46 CER, 6 months 38 IER and 43 CER

Table 4

Changes in insulin and related parameters over 6 months

Parameter		Baseline	1 Month	3 Month	6 Month	P value[4]
Insulin (μU/ml)[2]	IER	7.3 (6.3 to 8.4)	6.4 (5.7 to 7.3)	5.6 (4.7 to 6.5)	5.2[3] (4.5 to 6.0)	0.04
	CER	7.4 (6.4 to 8.6)	6.5 (5.7 to 7.5)	6.3 (5.4 to 7.3)	6.3[3] (5.4 to 7.4)	
HOMA (μU/mmol/L)[2]	IER	1.5 (1.3 to 1.8)	1.4 (1.2 to 1.6)	1.1 (1.0 to 1.4)	1.1[3] (0.9 to 1.3)	0.04
	CER	1.6 (1.3 to 1.8)	1.3 (1.2 to 1.6)	1.3 (1.1 to 1.5)	1.3[3] (1.1 to 1.6)	
Glucose (mmol/L)[1]	IER	4.8 (4.7 to 4.9)	4.8 (4.7 to 4.9)	4.7 (4.6 to 4.8)	4.7[3] (4.6 to 4.8)	0.34
	CER	4.8 (4.6 to 4.9)	4.7 (4.6 to 4.8)	4.7 (4.6 to 4.8)	4.7 (4.6 to 4.9)	
Adiponectin[2] (μg/ml)	IER	10.6 (9.5 to 11.8)	9.9 (8.8 to 11.0)	10.5 (9.3 to 11.9)	11.7[3] (10.3 to 13.4)	0.08
	CER	10.8 (9.7 to 12.1)	9.4 (8.3 to 10.6)	10.4 (9.1 to 11.9)	10.9 (9.7 to 12.3)	
Ghrelin (pg/ml)[2]	IER	136.0 (116.7 to 158.5)	159.4 (136.9 to 185.5)	167.8 (139.1 to 202.4)	153.3 (123.5 to 190.3)	0.92
	CER	132.5 (110.6 to 158.8	155.1 (130.8 to 184.0)	159.0 (131.4 to 192.3)	147.5 (120.7 to 180.3)	
BDNF (pg/ml)[1]	IER	9539 (8960 to 10118)	9435 (8890 to 9980)	9438 (8897 to 9978)	9214 (8722 to 9706)	0.87
	CER	9898 (9394 to 10402)	9606 (9144 to 10069)	9615 (9130 to 10101)	9528[3] (9093 to 9963)	
CRP (mg/L)[2]	IER	4.5 (3.8 to 5.4)	3.9 (3.3 to 4.6)	3.7 (3.0 to 4.4)	4.0[3] (3.3 to 4.8)	0.15
	CER	3.7 (3.2 to 4.3)	3.1 (2.7 to 3.5)	3.0 (2.6 to 3.4)	2.9[3] (2.5 to 3.4)	
Sialic acid (mg/L)[1]	IER	72.6 (70.3 to 75.0)	70.5 (67.9 to 73.1)	71.2 (68.7 to 73.7)	71.1 (68.3 to 73.9)	0.73
	CER	71.0 (68.6 to 73.3)	68.4 (65.9 to 70.9)	69.9 (67.6 to 72.2)	69.4 (66.8 to 71.9)	
AOPP fast acting (μM)[2]	IER	41.5 (34.8 to 49.5)	34.4 (29.7 to 39.9)	33.3 (28.2 to 39.3)	34.9[3] (30.1 to 40.4)	0.76
	CER	43.2 (36.7 to 51.0)	41.9 (35.4 to 49.7)	37.9 (32.9 to 43.7)	36.9[3] (31.5 to 43.2)	
AOPP aggregates, slow acting (μM)[2]	IER	1.7 (1.5 to 2.0)	1.8 (1.6 to 2.1)	1.8 (1.5 to 2.1)	1.6 (1.4 to 1.9)	0.12
	CER	1.4 (1.2 to 1.7)	1.6 (1.4 to 1.9)	1.6 (1.3 to 1.9)	1.7 (1.5 to 1.9)	
Ketones (μM)[2]	IER	40.8 (31.5 to 52.7)	77.1 (58.0 to 102.5)	73.0 (52.9 to 100.6)	67.6[3] (49.7 to 91.9)	0.12
	CER	48.0 (37.8 to 61.0)	71.1 (52.5 to 96.2)	63.3 (49.2 to 81.5)	49.6 (38.2 to 64.3)	

[1]Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months.

[2]Geometric Mean (95% CI) for baseline values and LOCF values at 1, 3 and 6 months

[3]Change from baseline to LOCF at 6 months within group is statistically significant $p<0.05$.

[4] Analysis of variance (ANOVA) for LOCF at 6 months between groups adjusted for baseline levels of each parameter, change in physical activity over 6 months and day of menstrual cycle.

IER = intermittent energy restriction, CER = continuous energy restriction Dietary intake data: Baseline 40 IER and 42 CER, 1 month 37 IER and 35 CER, 3 months 32 IER and 33 CER, 6 months 27 IER and 31 CER

Physical activity data: Baseline 50 IER and 52 CER, 1 month 49 IER and 47 CER, 3 months 42 IER and 46 CER, 6 months 38 IER and 43 CER

Table 5

Changes in risk markers for breast cancer and cardiovascular disease

Cardiovascular disease risk markers						
Parameter		**Baseline**	**1 Month**	**3 Month**	**6 Month**	**P value[4]**
Cholesterol (mmol/L)[1]	IER	5.1 (4.9 to 5.4)	4.6 (4.4 to 4.9)	4.8 (4.5 to 5.0)	4.8[3] (4.5 to 5.0)	0.62
	CER	5.2 (5.0 to 5.4)	4.8 (4.5 to 5.0)	4.8 (4.5 to 5.0)	4.7[3] (4.5 to 5.0)	
Triglycerides (mmol/L)[1]	IER	1.2 (1.0 to 1.4)	1.0 (0.9 to 1.2)	1.2 (0.9 to 1.5)	1.0[3] (0.9 to 1.2)	0.60
	CER	1.3 (1.1 to 1.4)	1.1 (0.9 to 1.3)	1.0 (0.9 to 1.1)	1.0[3] (0.8 to 1.2)	
HDL (mmol/L)[1]	IER	1.5 (1.4 to 1.5)	1.3 (1.2 to 1.4)	1.4 (1.3 to 1.5)	1.5 (1.4 to 1.6)	0.34
	CER	1.6 (1.4 to 1.7)	1.4 (1.3 to 1.5)	1.5 (1.3 to 1.6)	1.5[3] (1.4 to 1.6)	
LDL (mmol/L)[1]	IER	3.1 (2.9 to 3.3)	2.8 (2.6 to 3.1)	2.9 (2.6 to 3.1)	2.8[3] (2.6 to 3.1)	0.93
	CER	3.1 (2.8 to 3.3)	2.8 (2.6 to 3.0)	2.8 (2.6 to 3.1)	2.8[3] (2.6 to 3.0)	
BP systolic[1]	IER	115.2 (111.2 to 119.2)	111.6 (107.9 to 115.2)	110.2 (106.9 to 113.5)	111.5[3] (107.7 to 115.2)	0.99
	CER	116.8 (113.1 to 120.4)	110.0 (106.7 to 113.4)	110.9 (107.7 to 114.1)	109.3[3] (105.3 to 113.2)	
BP diastolic[1]	IER	76.7 (73.9 to 79.4)	72.6 (69.4 to 75.7)	72.2 (68.7 to 75.6)	72.4[3] (68.9 to 76.0)	0.84
	CER	75.4 (72.3 to 78.4)	71.1 (67.8 to 74.4)	70.5 (67.6 to 73.3)	69.7[3] (66.4 to 72.9)	
Breast cancer risk markers						
Leptin (ng/ml)[2]	IER	28.5 (23.2 to 35.0)	19.4 (15.5 to 24.4)	18.0 (14.2 to 122.8)	17.0[3] (13.4 to 21.5)	0.53
	CER	28.2 (23.5 to 33.8)	19.2 (15.3 to 24.2)	19.3 (15.7 to 23.8)	18.0[3] (14.1 to 22.8)	
Leptin/adiponectin[2] ratio ng/μg	IER	1.5 (1.3–1.6)	1.4 (1.2–1.5)	1.3 (1.2–1.4)	1.2 (1.1–1.4)	0.18
	CER	1.5 (1.3–1.6)	1.3 (1.2–1.5)	1.2 (1.1–1.4)	1.2 (1.0–1.3)	
Testosterone (nmol/L)[1]	IER	0.8 (0.7 to 0.9)	0.9 (0.8 to 1.0)	0.8 (0.7 to 0.9)	0.8 (0.7 to 0.9)	0.54
	CER	0.9 (0.8 to 1.0)	1.0 (0.8 to 1.1)	0.8 (0.7 to 0.9)	0.8 (0.7 to 0.9)	
Androstendione (μmol/L)[2]	IER	2.7 (2.4 to 3.0)	2.8 (2.4 to 3.1)	2.8 (2.5 to 3.1)	2.9 (2.6 to 3.2)	0.87
	CER	3.1 (2.8 to 3.4)	3.2 (2.9 to 3.6)	3.0 (2.7 to 3.4)	3.1 (2.8 to 3.4)	
DHEAS (μmol/L)[2]	IER	3.2 (2.8 to 3.7)	3.4 (2.9 to 3.9)	3.3 (2.9 to 3.8)	3.3 (2.8 to 3.8)	0.08
	CER	3.4 (3.0 to 3.8)	3.4 (3.1 to 3.9)	3.2 (2.8 to 3.6)	3.2[3] (2.8 to 3.6)	
SHBG (nmol/L)[2]	IER	43.2 (38.2 to 49.0)	49.3 (42.8 to 56.6)	48.6 (42.3 to 55.9)	49.2[3] (43.2 to 56.1)	0.21

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Cardiovascular disease risk markers						
Parameter		**Baseline**	**1 Month**	**3 Month**	**6 Month**	**P value[4]**
FAI (testosterone/[SHBG × 100])[2]	CER	42.0 (37.5 to 46.9)	46.1 (41.5 to 51.2)	44.3 (39.9 to 49.2)	44.6[3] (39.7 to 50.2)	0.90
	IER	1.7 (1.5 to 2.1)	1.6 (1.4 to 2.0)	1.6 (1.4 to 1.9)	1.6[3] (1.4 to 1.9)	
Prolactin (mIU/L)[1]	CER	2.0 (1.7 to 2.3)	2.0 (1.7 to 2.3)	1.8 (1.5 to 2.1)	1.8[3] (1.5 to 2.1)	0.98
	IER	269.6 (230.8 to 308.4)	244.2 (208.5 to 279.9)	244.0 (207.6 to 280.3)	267.1 (228.4 to 305.7)	
IGF-1 Total (μg/L)[2]	CER	245.3 (218.4 to 272.2)	259.6 (230.3 to 288.9)	270.6 (236.5 to 304.7)	257.4 (226.4 to 288.4)	0.17
	IER	201.3 (185.3 to 218.7)	210.8 (192.7 to 230.6)	207.7 (188.7 to 228.6)	191.6 (172.7 to 212.5)	
IGF-1 Free (μg/L)[1]	CER	202.9 (191.5 to 215.0)	212.9 (199.3 to 227.5)	211.4 (198.6 to 225.0)	203.7 (189.7 to 218.7)	0.71
	IER	0.7 (0.6 to 0.8)	-	-	0.6 (0.5 to 0.8)	
IGF BP-1 (μg/L)[2]	CER	0.6 (0.5 to 0.7)	-	-	0.6 (0.5 to 0.8)	0.74
	IER	21.4 (18.4 to 24.8)	23.3 (19.6 to 27.6)	26.3 (21.6 to 32.0)	27.0[3] (22.4 to 32.4)	
IGF BP-2 (μg/L)[2]	CER	22.6 (18.8 to 27.1)	22.7 (19.3 to 26.6)	25.4 (21.5 to 29.9)	29.0[3] (24.4 to 34.4)	0.13
	IER	108.8 (93.9 to 126.0)	125.6 (108.9 to 144.8)	140.2 (120.3 to 163.3)	148.4[3] (126.4 to 174.1)	
	CER	112.6 (99.2 to 127.8)	122.3 (105.6 to 141.6)	125.7 (109.9 to 143.7)	134.9[3] (115.8 to 157.2)	

[1] Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months.

[2] Geometric Mean (95% CI) for baseline values and LOCF values at 1, 3 and 6 months

[3] Change from baseline to LOCF at 6 months within group is statistically significant $p<0.05$.

[4] Analysis of variance (ANOVA) for LOCF at 6 months between groups adjusted for baseline levels of each parameter, change in physical activity over 6 months and day of menstrual cycle.

IER = intermittent energy restriction, CER = continuous energy restriction Baseline 53 IER and 54 CER, 1 month 51 IER and 51 CER, 3 months 45 IER and 47 CER, 6 months 42 IER and 47 CER