International Journal of Obesity (2011) 35, 714–727

0307-0565/11
www.nature.com/ijo

ORIGINAL ARTICLE

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

MN Harvie[1], M Pegington[1], MP Mattson[2], J Frystyk[3], B Dillon[4], G Evans[1], J Cuzick[5], SA Jebb[6], B Martin[2], RG Cutler[2], TG Son[2], S Maudsley[2], OD Carlson[2], JM Egan[2], A Flyvbjerg[3] and A Howell[1]

[1]*Genesis Prevention Centre, University Hospital of South Manchester NHS Foundation Trust, Manchester, UK;* [2]*Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Baltimore, USA;* [3]*Clinical Institute of Medicine & Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark;* [4]*Department of Statistics University Hospital of South Manchester, Manchester, UK;* [5]*CRUK Centre for Epidemiology, Mathematics and Statistics, Wolfson Institute of Preventive Medicine, Queen Mary's School of Medicine, London, UK and* [6]*MRC Human Nutrition Research Group, Cambridge, UK*

Background: The problems of adherence to energy restriction in humans are well known.
Objective: To compare the feasibility and effectiveness of intermittent continuous energy (IER) with continuous energy restriction (CER) for weight loss, insulin sensitivity and other metabolic disease risk markers.
Design: Randomized comparison of a 25% energy restriction as IER (~2710 kJ/day for 2 days/week) or CER (~6276 kJ/day for 7 days/week) in 107 overweight or obese (mean (±s.d.) body mass index 30.6 (±5.1) kg m^{-2}) premenopausal women observed over a period of 6 months. Weight, anthropometry, biomarkers for breast cancer, diabetes, cardiovascular disease and dementia risk; insulin resistance (HOMA), oxidative stress markers, leptin, adiponectin, insulin-like growth factor (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 that IER and CER are equally effective for weight loss: mean (95% confidence interval) 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 with CER; difference between groups for fasting insulin was −1.2 (−1.4 to −1.0) μU ml^{-1} and for insulin resistance was −1.2 (−1.5 to −1.0) μU mmol^{-1} l^{-1} (both $P = 0.04$).
Conclusion: IER is as effective as CER with regard 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.
International Journal of Obesity (2011) **35,** 714–727; doi:10.1038/ijo.2010.171; published online 5 October 2010

Keywords: intermittent; continuous energy restriction; randomized; premenopausal women; insulin sensitivity

Introduction

Excess weight and weight gain during adult life increase the risk of several diseases, including diabetes,[1] cardiovascular disease,[2] dementia[3] and certain forms of cancer including breast cancer,[4] and can contribute to premature death.[5] Observational and some randomized trials indicate that modest weight reduction (>5% of body weight) reduces the

Correspondence: Dr MN Harvie, Senior Research Dietitian, Nightingale Centre and Genesis Prevention Centre, University Hospital of South Manchester NHS Foundation Trust, Manchester M23 9LT, UK.
E-mail: michelle.harvie@manchester.ac.uk
Received 5 May 2010; revised 15 July 2010; accepted 19 July 2010; published online 5 October 2010

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 when reduced weights are maintained, many of the benefits achieved during weight loss, including improvements in insulin sensitivity, may be attenuated because of 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 fasting every other day to 3 weeks of partial energy restriction and refeeding. In these studies, IER seems to be 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 the lifespan of rodents.[18] IER may even produce benefits similar to those observed following more stringent CER.[14] Few human studies have examined the effects of IER, possibly because of concerns of disordered eating patterns and overconsumption on non-restricted days. Several short-term studies suggest that this does not occur.[19,20] We report a randomized trial of 25% energy restriction delivered as IER vs 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 30–45 years with adult weight gain exceeding 10 kg since the age of 20 years, and a body mass index 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) (the 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 per week) or phyto-oestrogens, and were not suffering from diagnosed diabetes, cardiovascular disease, major psychiatric morbidity or cancer. We solicited participants from our Family History Clinic by mail shot, and women in the general population using 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) before recruitment. Of the 135 who were eligible after screening, 13 (9%) did not believe they could tolerate the diet for the 6-month trial period, and a further 14 (10%) decided not to participate because of 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 body mass index (above or below the predicted median value 28 kg m^{-2}), family history of breast cancer, sedentary lifestyle (either $<$ or >1 h moderate activity per week) and also according to the evaluating study dietitian to ensure that the two 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 for 7 days per week, or the IER of 25% restriction delivered as a VLCD for 2 days per week, with no restriction on the other 5 days per week.

Measurements were made before starting and at 1, 3 and 6 months. These included weight, total body fat, fat-free mass determined by impedance (Tanita TBF-300A, Tanita Europe BV, Yiewsley, UK) waist, hip, bust and thigh circumference, systolic and diastolic blood pressure (Omron M5-1 Omron Healthcare Limited, Milton Keynes, UK) and blood sampling. All assessments were conducted in the morning after a 12-h fast. Weight and body fat were assessed wearing light clothing. Body circumferences were measured in triplicate according to study protocols.[23] Blood pressure was measured in triplicate after 10 min at rest and the mean value was calculated. The IER group was 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] However, additional fasting serum samples were 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 was asked to record

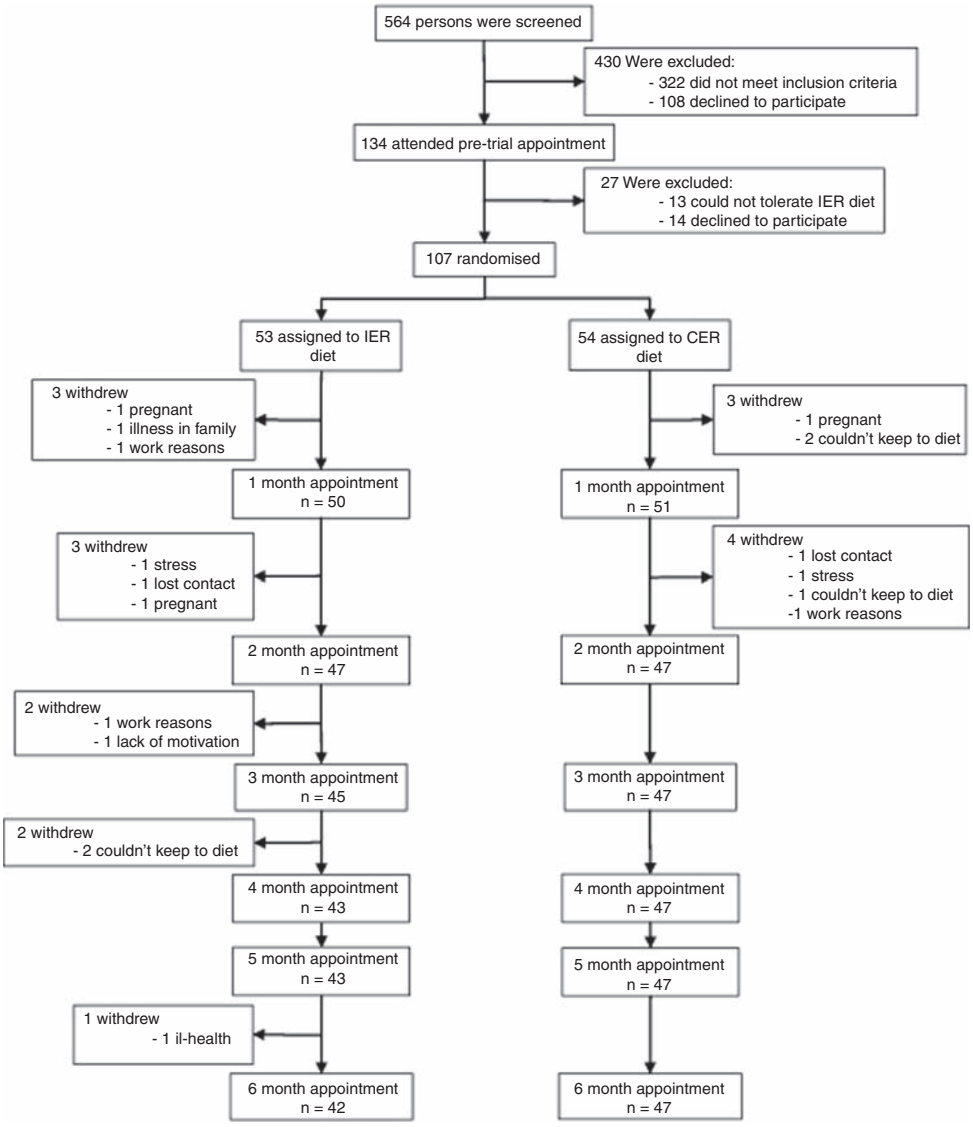

Figure 1 Uptake and recruitment to trial.

whether they had successfully completed a 2-, 1- or 0-day 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 as the number of IER subjects reporting 2- or 1-day VLCD each week and the number of CER subjects achieving a 25% energy restriction. Physical activity was assessed using the validated international physical activity questionnaire expressed as metabolic energy turnover in minutes per day and kJ per 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 the day of the 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 metabolic energy turnovers $\times$ estimated basal metabolic rate.[28]

The CER group was prescribed a daily 25% restriction based on a Mediterranean-type diet (30% fat, 15% mono-unsaturated, 7% saturated fat, 7% polyunsaturated fatty acids, 45% low glycaemic load carbohydrate and 25%

protein).[29] The IER group was asked to undertake a VLCD (75% restriction) on two consecutive days and to consume estimated requirements for weight maintenance for the remaining 5 days according to the nutrient composition above. The VLCD provided 2700 kJ of energy and 50 g protein per day and comprised 1.136 l (two pints) of semi-skimmed milk, four portions of vegetables (~80 g per portion), one 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 individualized food portion lists, meal plans and recipes. To maximize compliance, patients received fortnightly motivational phone calls and monthly clinical appointments, in which 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 the University Hospital of South Manchester NHS Foundation Trust using the following methods: insulin using electrochemoluminescence immunoassay (Elecsys Roche Diagnostics, Lewes, UK, within-batch coefficient of variation (CV) 1.9%); glucose using hexokinase/glucose-6-phosphate interassay dehydrogenase method (Bayer, Newbury, UK, CV 3%); sex hormone binding globulin (SHBG) using non-competitive IRMA (IRMA-Orion Diagnostica Oy, Espoo, Finland, CV 2.7%), prolactin using electrochemoluminescence immunoassay (Elecsys Roche Diagnostics, 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 homoeostasis model assessment,[31] whereas free androgen index was also estimated by the equation 100 × serum testosterone/serum SHBG.[32] Colorimetric enzyme reactions were used to measure total cholesterol (CV 0.8%), triglycerides (CV 1.5%) and high-density lipoprotein cholesterol (CV 1.0%) (all Roche Modular E170, Roche, Welwyn Garden City, UK). Levels were measured spectrophotometrically by an automated Olympus AU600 analyser. Low-density cholesterol was calculated using the formula of Friedewald *et al*.[33] Adipokines leptin and adiponectin and inflammatory markers high-sensitivity C-reactive protein 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, Minneapolis, MN, USA, Quantikine Human Leptin kit, R&D Systems; CV 10%), whereas plasma adiponectin was measured using radioimmunoassay (LINCO Research, St Charles, MO, 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; CV 1.2%) adapted for use on the Hitachi 912 Clinical Analyser (Roche) and high-sensitivity C-reactive protein using a high-sensitivity particle enhanced turbidometric assay (Dade-Behring, Walton, UK; CV 4.5%).

Total insulin-like growth factor (IGF)-1 (CV 3.2%), ultrafiltered 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 (β-hydroxybutyric acid (~80%) and acetoacetone) (CV 1.6%), brain-derived neurotrophic factor (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 were measured using a modified method of Selmeci *et al*.[39] (CV 2.2%). All serum and plasma samples were stored at 4 °C for no longer than 4 h, aliquoted and frozen at −70 °C within 24 h 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 (CIs)) or geometric mean (95% CI) for the log-transformed variables (fasting insulin, insulin resistance, adiponectin, high-sensitivity C-reactive protein, total IGF-1, IGFBP-1, IGFBP-2, ghrelin, total ketone bodies, fast- and slow-acting advanced oxidation protein products, androstenedione, DHEAS, SHBG, free androgen index, leptin, leptin:adiponectin ratio and physical activity (metabolic energy turnover in min/day and kJ/day)).

The primary aim of this 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% dropout. The primary analysis was a last observation carried forward (LOCF) analysis of variance at 6 months between the groups defined at randomization 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 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, Chicago, IL, USA).

Changes in weight, body fat, waist and insulin resistance, over the trial period, were also measured using generalized estimating equations to allow all three time points to be analysed simultaneously, and to incorporate data from subjects with less than three time points without the need for substitution, thus increasing statistical power and a more efficient comparison across the various time points. These generalized estimating equations models were constructed in Stata 10 (StataCorp LP, College Station, TX, USA) with an exchangeable correlation structure; the predictors used were the three time points (1, 3, 6 months), the group variable (IER vs CER) and 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 comorbidities, which were equally frequent in the two groups. In total, 6 IER (11%) and 10 CER (18%) met the Diabetes Federation Criteria for metabolic syndrome.[41] The majority of subjects reported previous attempts at dieting (IER 92%, CER 78%), with a comparable number of 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 ($\chi^2=1.16$, $P=0.28$). The main reasons for dropout 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 (77.5–85.4) to 75 (71.2–78.8) kg in the IER group compared with a reduction from 84.4 (79.7–89.1) to 78.7 (74.2–83.2) 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 ($\chi^2=1.89$, $P=0.39$). Both groups experienced comparable reductions in body fat, fat-free mass, hip, bust and thigh circumference and composition of weight loss. The 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). Generalized estimating equations 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 nonsignificant greater decline in waist measurement with IER at 3 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).

Table 1 Baseline characteristics of subjects

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

Abbreviations: BMI, body mass index; CER, continuous energy restriction; IER, intermittent energy restriction. [a]Mean (s.d.), Independent sample *t*-test. [b]Tyrer–Cuzick model.[21] [c]*N* (%), χ^2 test.

Adherence

Weekly dietary records were available for 82 (76%) subjects at baseline, for 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)%, ($P=0.004$).

Table 2 Change in weight and circumferences over 6 months

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

Abbreviations: IER, intermittent energy restriction, CER, continuous energy restriction. [a]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. [b]Change from baseline to LOCF is statistically significant at 6 months within group $P<0.05$ Mean (95% CI) for baseline and last observation carried forward (LOCF) values at 1, 3 and 6 months. 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.

Intention-to-treat analysis assuming that women who left the study or who did not complete food diaries did not adhere to the diets shows reported adherence to 2-day VLCD among 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 1 day of VLCD at 1, 3 and 6 months, respectively. The proportion of CER subjects who reported to adhere 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-day 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, whereas 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 randomization. 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 among the IER group (Table 4). Mean difference between groups (95% CI) for fasting insulin was −1.2 (−1.4 to −1.0) $\mu U\,ml^{-1}$, −16 (−19 to −13)%, ($P=0.04$); and for insulin resistance was −1.2 (−1.5 to −1.0) $\mu U\,mmol^{-1}\,l^{-1}$, −45 (−86 to −3)%, ($P=0.04$) (Table 4). Generalized estimating equations 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 in 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 were observed in total and free IGF-1.

Both groups experienced modest decreases in the inflammatory marker high-sensitivity C-reactive protein, but no change in sialic acid levels. The groups had comparable reductions in the oxidative stress marker, fast-acting advanced oxidation protein products, by 6 months, which appeared to occur earlier in IER compared with CER. Slow-acting advanced

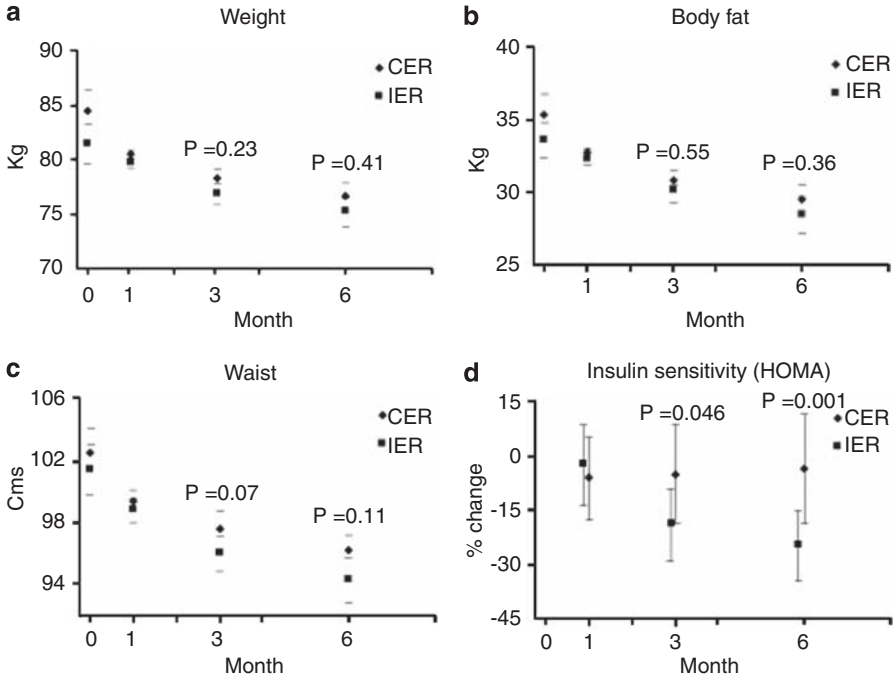

Figure 2 GEE modelling of changes in weight, body fat, waist and insulin sensitivity (HOMA) with intermittent energy restriction ($N=53$) and continuous energy restriction ($N=54$) over 6 months.

oxidation protein products appeared to decrease in the IER group and to have a slight increase in the CER group (mean difference between groups at 6 months (95% CI) −10 (−19 to 2)%, $P=0.12$). Women in the IER group had a nonsignificant greater increase in serum total ketone bodies at 6 months compared with 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 brain-derived neurotrophic factor or for fasting glucose.

Breast cancer risk markers

Both groups experienced large reductions in serum leptin, decreases in the ratio of leptin:adiponectin, and no changes in serum levels of testosterone, androstenedione and prolactin. The CER group had a greater reduction in DHEAS compared with 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 free androgen index (Table 5). Menstrual cycle data were available for 44 IER (83%) and 47 CER (87%) subjects. During the 6-month study period, the mean (±s.d.) length of menstrual cycle was significantly longer in the IER group compared with 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 low-density lipoprotein cholesterol, triglycerides, systolic and diastolic BP. Neither group experienced changes in high-density lipoprotein levels (Table 5).

Effects of IER and CER on serum markers over 1 week

A subset 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%), homoeostasis model assessment (−29%) and triglycerides (−18%) in the morning after the 2-day VLCD, which normalized within 2 days of resuming a 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 subjects in the IER group (4, 8%), but none in the CER group, experienced minor adverse physical symptoms, including lack of energy, headache, feeling cold and constipation. Eight (15%) of the IER and none of the CER subjects complained of hunger, whereas a further three (6%) of the IER and seven (13%) subjects of the CER group reported increased energy and improved health. Around 8 (15%) subjects 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, whereas 17 (32%) of the IER and 25 (46%) of the CER group reported increased self-confidence and a positive mood. Predictably, both groups acknowledged the limited food choice of the diets: 55% IER and 53% CER. More subjects of

Table 3 Changes in dietary intake and physical activity over 6 months

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

Abbreviations: IER, intermittent energy restriction; CER, continuous energy restriction. [a]Analysis of variance (ANOVA) for (last observation carried forward) LOCF at 6 months between groups adjusted for baseline levels of each parameter. [b]Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months. [c]Change from baseline to LOCF at 6 months within group is statistically significant within group $P<0.05$. [d]Geometric mean (95% CI) for baseline values and LOCF values at 1, 3 and 6 months. 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.

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%), for 91 at 1 and 3 months (84%) and for 75 at 6 months (69%). There was a modest increase in the physical component summary score in the IER but not in the CER group (mean difference (95% CI) 2.1 (−0.1 to 4.3) units, 4 (0.0–8.0)%, $P=0.06$). In comparison, there was a slightly greater increase in the mental component summary score in the CER compared with the IER group (2.8 (0.1–5.6) units, 5 (0.0–12.0)%, $P=0.04$).

Discussion

Main findings

This is the largest randomized 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, IGFBP1 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 weeks) randomized studies have reported the effects of IER vs CER. Ash *et al*.[20] compared an IER (4180 kJ liquid VLCD 4 days per week, 3 days *ad libitum*) vs CER (6000–7000 kJ/day) among nine men with type 2 diabetes and showed no difference in terms of weight or

Table 4 Changes in insulin and related parameters over 6 months

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

Abbreviations: IER, intermittent energy restriction; CER, continuous energy restriction. [a]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. [b]Geometric mean (95% CI) for baseline values and LOCF values at 1, 3 and 6 months. [c]Change from baseline to LOCF at 6 months within group is statistically significant $P<0.05$. [d]Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months. 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.

fasting insulin. Hill *et al*.[19] compared alternating weeks of 2508, 3762, 5016 or 7254 kJ/day as compared with constant restriction of 5016 kJ/day in 16 moderately obese women and reported greater reductions in cholesterol in the IER group compared with the CER group (−14 vs −6%). A further study among patients with type 2 diabetes showed beneficial effects of periodic VLCD (either 1 day per week or 5 consecutive days every 5 weeks), in addition to, and not instead of, a normal daily restriction (6180–7416 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

Table 5 Changes in risk markers for breast cancer and cardiovascular disease

Parameter	*Baseline*	*1 month*	*3 months*	*6 months*	P-*value*[a]
Cardiovascular disease risk markers					
Cholesterol (mmol l^{-1})[b]					
IER	5.1 (4.9–5.4)	4.6 (4.4–4.9)	4.8 (4.5–5.0)	4.8 (4.5–5.0)[c]	0.62
CER	5.2 (5.0–5.4)	4.8 (4.5–5.0)	4.8 (4.5–5.0)	4.7 (4.5–5.0)[c]	
Triglycerides (mmol l^{-1})[b]					
IER	1.2 (1.0–1.4)	1.0 (0.9–1.2)	1.2 (0.9–1.5)	1.0 (0.9–1.2)[c]	0.60
CER	1.3 (1.1–1.4)	1.1 (0.9–1.3)	1.0 (0.9–1.1)	1.0 (0.8–1.2)[c]	
HDL (mmol l^{-1})[b]					
IER	1.5 (1.4–1.5)	1.3 (1.2–1.4)	1.4 (1.3–1.5)	1.5 (1.4–1.6)	0.34
CER	1.6 (1.4–1.7)	1.4 (1.3–1.5)	1.5 (1.3–1.6)	1.5 (1.4–1.6)[c]	
LDL (mmol l^{-1})[b]					
IER	3.1 (2.9–3.3)	2.8 (2.6–3.1)	2.9 (2.6–3.1)	2.8 (2.6–3.1)[c]	0.93
CER	3.1 (2.8–3.3)	2.8 (2.6–3.0)	2.8 (2.6–3.1)	2.8 (2.6–3.0)[c]	
BP systolic (mm Hg)[b]					
IER	115.2 (111.2–119.2)	111.6 (107.9–115.2)	110.2 (106.9–113.5)	111.5 (107.7–115.2)[c]	0.99
CER	116.8 (113.1–120.4)	110.0 (106.7–113.4)	110.9 (107.7–114.1)	109.3 (105.3–113.2)[c]	
BP diastolic (mm Hg)[b]					
IER	76.7 (73.9–79.4)	72.6 (69.4–75.7)	72.2 (68.7–75.6)	72.4 (68.9–76.0)[c]	0.84
CER	75.4 (72.3–78.4)	71.1 (67.8–74.4)	70.5 (67.6–73.3)	69.7 (66.4–72.9)[c]	
Breast cancer risk markers					
Leptin (ng ml^{-1})[d]					
IER	28.5 (23.2–35.0)	19.4 (15.5–24.4)	18.0 (14.2–122.8)	17.0 (13.4–21.5)[c]	0.53
CER	28.2 (23.5–33.8)	19.2 (15.3–24.2)	19.3 (15.7–23.8)	18.0 (14.1–22.8)[c]	
Leptin/adiponectin ratio (ng μg^{-1})[d]					
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})[b]					
IER	0.8 (0.7–0.9)	0.9 (0.8–1.0)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.54
CER	0.9 (0.8–1.0)	1.0 (0.8–1.1)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	
Androstendione (μmol l^{-1})[d]					
IER	2.7 (2.4–3.0)	2.8 (2.4–3.1)	2.8 (2.5–3.1)	2.9 (2.6–3.2)	0.87
CER	3.1 (2.8–3.4)	3.2 (2.9–3.6)	3.0 (2.7–3.4)	3.1 (2.8–3.4)	
DHEAS (μmol l^{-1})[d]					
IER	3.2 (2.8–3.7)	3.4 (2.9–3.9)	3.3 (2.9–3.8)	3.3 (2.8–3.8)	0.08
CER	3.4 (3.0–3.8)	3.4 (3.1–3.9)	3.2 (2.8–3.6)	3.2 (2.8–3.6)[c]	
SHBG (nmol l)[d]					
IER	43.2 (38.2–49.0)	49.3 (42.8–56.6)	48.6 (42.3–55.9)	49.2 (43.2–56.1)[c]	0.21
CER	42.0 (37.5–46.9)	46.1 (41.5–51.2)	44.3 (39.9–49.2)	44.6 (39.7–50.2)[c]	
FAI (testosterone/(SHBG × 100))[d]					
IER	1.7 (1.5–2.1)	1.6 (1.4–2.0)	1.6 (1.4–1.9)	1.6 (1.4–1.9)[c]	0.90
CER	2.0 (1.7–2.3)	2.0 (1.7–2.3)	1.8 (1.5–2.1)	1.8 (1.5–2.1)[c]	
Prolactin (mIU l^{-1})[b]					
IER	269.6 (230.8–308.4)	244.2 (208.5–279.9)	244.0 (207.6–280.3)	267.1 (228.4–305.7)	0.98
CER	245.3 (218.4–272.2)	259.6 (230.3–288.9)	270.6 (236.5–304.7)	257.4 (226.4–288.4)	
IGF-1 total (μg l^{-1})[d]					
IER	201.3 (185.3–218.7)	210.8 (192.7–230.6)	207.7 (188.7–228.6)	191.6 (172.7–212.5)	0.17
CER	202.9 (191.5–215.0)	212.9 (199.3–227.5)	211.4 (198.6–225.0)	203.7 (189.7–218.7)	
IGF-1 free (μg l^{-1})[b]					
IER	0.7 (0.6–0.8)	—	—	0.6 (0.5–0.8)	0.71
CER	0.6 (0.5–0.7)	—	—	0.6 (0.5–0.8)	
IGF BP-1 (μg l^{-1})[d]					
IER	21.4 (18.4–24.8)	23.3 (19.6–27.6)	26.3 (21.6–32.0)	27.0 (22.4–32.4)[c]	0.74
CER	22.6 (18.8–27.1)	22.7 (19.3–26.6)	25.4 (21.5–29.9)	29.0 (24.4–34.4)[c]	
IGF BP-2 (μg l^{-1})[d]					
IER	108.8 (93.9–126.0)	125.6 (108.9–144.8)	140.2 (120.3–163.3)	148.4 (126.4–174.1)[c]	0.13
CER	112.6 (99.2–127.8)	122.3 (105.6–141.6)	125.7 (109.9–143.7)	134.9 (115.8–157.2)[c]	

Abbreviations: BP, blood pressure; CER, continuous energy restriction; DHEAS, dehydroepiandrosterone sulphate; IER, intermittent energy restriction; IGF, insulin-like growth factor, FAI, free androgen index, HDL, high-density lipoprotein; LDL, low-density lipoprotein; SHBG, sex hormone binding globulin. [a]Analysis of variance (ANOVA) for last observation carried forward (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. [b]Mean (95% CI) for baseline and LOCF values at 1, 3 and 6 months. [c]Change from baseline to LOCF at 6 months within group is statistically significant $P<0.05$. [d]Geometric mean (95% CI) for baseline values and LOCF values at 1, 3 and 6 months. 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.

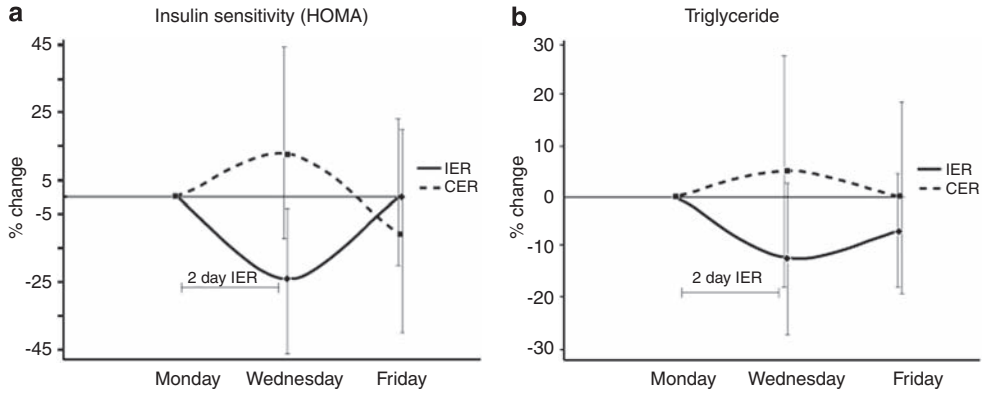

Figure 3 Changes in insulin sensitivity and triglycerides over the week with intermittent energy restriction ($N=15$) and continuous energy restriction ($N=9$).

our population, which was not particularly insulin resistant (16% of subjects met the Diabetes Federation Criteria for the metabolic syndrome), is not known. IER seemed to bring about a modest increase in adiponectin, which has a pivotal role in insulin sensitivity and in 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 among 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] Brain-derived neurotrophic factor is upregulated 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 among overweight asthmatics,[38] but increased levels among healthy overweight subjects.[47] In our study, both CER and IER led to anticipated increases in serum levels of ghrelin.[48]

Reductions in circulating sex steroid levels may reduce risk of breast cancer. The declines in free androgen index observed in both groups have been reported previously in premenopausal women.[49] The greater reduction in DHEAS with CER may be advantageous and may translate into greater reductions in breast cancer risk in women,[50] in contrast to men, in whom higher levels of DHEAS are linked to longevity.[51] Conversely, the greater average cycle length among 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 because of 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 with 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 subjects achieved >5% weight loss), which may reflect the motivation of the participants and ongoing monitoring and motivational calls. A number of subjects of the IER group experienced minor adverse physical and mental symptoms with 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 per day) reported no adverse effects on cognition, energy levels, sleep or mood,[56] suggesting that 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 h fast among healthy weight subjects.[57]

Strengths of this study

Previously reported weight loss and benefits of intermittent restriction have been reported from single-arm studies.[38,58] Our randomized 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

indicate that 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 per week. We believe this to be more achievable than previously studied regimens of alternate-day fasting or VLCD.[38,58,59] We tested the diets among overweight and obese free-living individuals, as 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 that acceptability of intermittent VLCD may be greater among men than among women.[42,60]

Study limitations

Although 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 subjects of the IER group (58%) planned to continue with the regimen beyond 6 months compared with 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 the 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 homoeostasis model assessment, which is an accepted method among non-diabetics.[31] Future trials should, however, compare the effects of IER with 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 with CER, could also be ascertained from measuring HBA1c and fructosamine. Such studies could also examine the relative effects 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 suggest 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.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Michelle N Harvie: study conception and design, trial management and manuscript preparation. Mary Pegington: running the trial, statistical analysis and manuscript preparation. Mark P Mattson: consultation, assays at NIH and manuscript preparation. Jan Frystyk and Allan Flyvbjerg: consultation, IGF-1 assays and manuscript preparation. Bernice Dillon: GEE modelling. Jack Cuzick: design of the trial, statistical advice and manuscript preparation. Gareth Evans: recruitment of subjects, consultation and manuscript preparation. Susan Jebb: MRC HNR assays, consultation and manuscript preparation. Bronwen Martin: BDNF and ghrelin assays and manuscript preparation. Roy G Cutler: AOPP and total ketone bodies assays. Tae G Son: AOPP and total ketone bodies assays. Stuart Maudsley: BDNF and ghrelin assay validation. Olga D Carlson: technical assistance with NIH assays. Josephine M Egan: BDNF and ghrelin assay validation and manuscript preparation. Anthony Howell: study conception and design, manuscript preparation. We thank Julie Morris for her invaluable statistical advice, Helen Sumner for coordinating sample storage and processing, Rosemary Greenhaugh and Jenny Affen for assisting in recruitment and sample collection, Emma Campbell for quality-of-life analysis, Lorraine Darmody, Angela Foster, Jane Eaton and Philippa Quirk for clerical support, Padraig McQuaid for help with dietary analysis. Aram Rudenski for advice on the HOMA model. We dedicate this paper to Andrew Shenton (database manager) who died tragically at a young age on 19 February 2008. Funding*:* Breast Cancer Campaign, World Cancer Research Fund, Genesis Appeal Manchester UK, Intramural Research Program of the National Institute on Aging of the NIH, the Danish Research Council for Health and Disease, Tanita Europe BV Middlesex UK for provision of Tanita TBF-300.

References

1 Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med* 1995; **122**: 481–486.
2 Willett WC, Manson JE, Stampfer MJ, Colditz GA, Rosner B, Speizer FE *et al*. Weight, weight change, and coronary heart disease in women. Risk within the 'normal' weight range. *JAMA* 1995; **273**: 461–465.

3 Beydoun MA, Beydoun HA, Wang Y. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. *Obes Rev* 2008; **9**: 204–218.
4 Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; **371**: 569–578.
5 Peeters A, Barendregt JJ, Willekens F, Mackenbach JP, Al Mamun A, Bonneux L. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Ann Intern Med* 2003; **138**: 24–32.
6 Harvie M, Howell A, Vierkant RA, Kumar N, Cerhan JR, Kelemen LE *et al*. Association of gain and loss of weight before and after menopause with risk of postmenopausal breast cancer in the Iowa women's health study. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 656–661.
7 Lindstrom J, Uusitupa M. Lifestyle intervention, diabetes, and cardiovascular disease. *Lancet* 2008; **371**: 1731–1733.
8 Chlebowski RT, Blackburn GL, Thomson CA, Nixon DW, Shapiro A, Hoy MK *et al*. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. *J Natl Cancer Inst* 2006; **98**: 1767–1776.
9 Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *JAMA* 2005; **293**: 43–53.
10 Henry RR, Scheaffer L, Olefsky JM. Glycemic effects of intensive caloric restriction and isocaloric refeeding in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1985; **61**: 917–925.
11 Kelley DE, Wing R, Buonocore C, Sturis J, Polonsky K, Fitzsimmons M. Relative effects of calorie restriction and weight loss in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1993; **77**: 1287–1293.
12 Anson RM, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A *et al*. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc Natl Acad Sci USA* 2003; **100**: 6216–6220.
13 Cleary MP, Jacobson MK, Phillips FC, Getzin SC, Grande JP, Maihle NJ. Weight-cycling decreases incidence and increases latency of mammary tumors to a greater extent than does chronic caloric restriction in mouse mammary tumor virus-transforming growth factor-alpha female mice. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 836–843.
14 Berrigan D, Perkins SN, Haines DC, Hursting SD. Adult-onset calorie restriction and fasting delay spontaneous tumorigenesis in p53-deficient mice. *Carcinogenesis* 2002; **23**: 817–822.
15 Bonorden MJ, Rogozina OP, Kluczny CM, Grossmann ME, Grande JP, Lokshin A *et al*. Cross-sectional analysis of intermittent versus chronic caloric restriction in the TRAMP mouse. *Prostate* 2009; **69**: 317–326.
16 Halagappa VK, Guo Z, Pearson M, Matsuoka Y, Cutler RG, Laferla FM *et al*. Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* 2007; **26**: 212–220.
17 Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem* 2005; **16**: 129–137.
18 Sogawa H, Kubo C. Influence of short-term repeated fasting on the longevity of female (NZB x NZW)F1 mice. *Mech Ageing Dev* 2000; **115**: 61–71.
19 Hill JO, Schlundt DG, Sbrocco T, Sharp T, Pope-Cordle J, Stetson B *et al*. Evaluation of an alternating-calorie diet with and without exercise in the treatment of obesity. *Am J Clin Nutr* 1989; **50**: 248–254.
20 Ash S, Reeves MM, Yeo S, Morrison G, Carey D, Capra S. Effect of intensive dietetic interventions on weight and glycaemic control in overweight men with Type II diabetes: a randomised trial. *Int J Obes Relat Metab Disord* 2003; **27**: 797–802.
21 Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. *Stat Med* 2004; **23**: 1111–1130.
22 Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W *et al*. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab* 2006; **91**: 4237–4245.
23 Seiddell J. Waist/hip and waist/thigh ratios. In: Fidanza (ed). Chapman and Hall: London, 1991, pp 24–29.
24 Ekelund U, Sepp H, Brage S, Becker W, Jakes R, Hennings M *et al*. Criterion-related validity of the last 7-day, short form of the International Physical Activity Questionnaire in Swedish adults. *Public Health Nutr* 2006; **9**: 258–265.
25 Hays RD, Morales LS. The RAND-36 measure of health-related quality of life. *Ann Med* 2001; **33**: 350–357.
26 Anttila L, Koskinen P, Irjala K, Kaihola HL. Reference intervals for serum sex steroids and gonadotropins in regularly menstruating women. *Acta Obstet Gynecol Scand* 1991; **70**: 475–481.
27 Tonolo G, Ciccarese M, Brizzi P, Milia S, Dessole S, Puddu L *et al*. Cyclical variation of plasma lipids, apolipoproteins, and lipoprotein(a) during menstrual cycle of normal women. *Am J Physiol* 1995; **269** (6 Pt 1): E1101–E1105.
28 Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* 1985; **39** (Suppl 1): 5–41.
29 Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I *et al*. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med* 2008; **359**: 229–241.
30 Avenell A, Sattar N, Lean M. ABC of obesity. Management: Part I—behaviour change, diet, and activity. *BMJ* 2006; **333**: 740–743.
31 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
32 Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999; **84**: 3666–3672.
33 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.
34 Chen DC, Chung YF, Yeh YT, Chaung HC, Kuo FC, Fu OY *et al*. Serum adiponectin and leptin levels in Taiwanese breast cancer patients. *Cancer Lett* 2006; **237**: 109–114.
35 Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B *et al*. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. *Diabetologia* 2009; **52**: 2345–2349.
36 Frystyk J, Skjaerbaek C, Dinesen B, Orskov H. Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Lett* 1994; **348**: 185–191.
37 Krassas GE, Pontikides N, Kaltsas T, Dumas A, Frystyk J, Chen JW *et al*. Free and total insulin-like growth factor (IGF)-I, -II, and IGF binding protein-1, -2, and -3 serum levels in patients with active thyroid eye disease. *J Clin Endocrinol Metab* 2003; **88**: 132–135.
38 Johnson JB, Summer W, Cutler RG, Martin B, Hyun DH, 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**: 665–674.
39 Selmeci L, Seres L, Antal M, Lukacs J, Regoly-Merei A, Acsady G. Advanced oxidation protein products (AOPP) for monitoring oxidative stress in critically ill patients: a simple, fast and inexpensive automated technique. *Clin Chem Lab Med* 2005; **43**: 294–297.
40 Tworoger SS, Yasui Y, Chang L, Stanczyk FZ, McTiernan A. Specimen allocation in longitudinal biomarker studies: controlling

subject-specific effects by design. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 1257–1260.
41 Alberti KG, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; **23**: 469–480.
42 Williams KV, 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.
43 Bar RS, Gorden P, Roth J, Kahn CR, De Meyts P. Fluctuations in the affinity and concentration of insulin receptors on circulating monocytes of obese patients: effects of starvation, refeeding, and dieting. *J Clin Invest* 1976; **58**: 1123–1135.
44 Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. *Diabetes Obes Metab* 2007; **9**: 282–289.
45 Halberg N, Henriksen M, Soderhamn 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**: 2128–2136.
46 Varady KA, Roohk DJ, Hellerstein MK. Dose effects of modified alternate-day fasting regimens on *in vivo* cell proliferation and plasma insulin-like growth factor-1 in mice. *J Appl Physiol* 2007; **103**: 547–551.
47 Araya AV, Orellana X, Espinoza J. Evaluation of the effect of caloric restriction on serum BDNF in overweight and obese subjects: preliminary evidences. *Endocrine* 2008; **33**: 300–304.
48 Hayes MR, Miller CK, Ulbrecht JS, Mauger JL, Parker-Klees L, Gutschall MD *et al*. A carbohydrate-restricted diet alters gut peptides and adiposity signals in men and women with metabolic syndrome. *J Nutr* 2007; **137**: 1944–1950.
49 Turcato E, Zamboni M, De Pergola G, Armellini F, Zivelonghi A, Bergamo-Andreis IA *et al*. Interrelationships between weight loss, body fat distribution and sex hormones in pre- and postmenopausal obese women. *J Intern Med* 1997; **241**: 363–372.
50 Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 2002; **94**: 606–616.
51 Enomoto M, Adachi H, Fukami A, Furuki K, Satoh A, Otsuka M *et al*. Serum dehydroepiandrosterone sulfate levels predict longevity in men: 27-year follow-up study in a community-based cohort (Tanushimaru study). *J Am Geriatr Soc* 2008; **56**: 994–998.
52 Alvero R, Kimzey L, Sebring N, Reynolds J, Loughran M, Nieman L *et al*. Effects of fasting on neuroendocrine function and follicle development in lean women. *J Clin Endocrinol Metab* 1998; **83**: 76–80.
53 Kok P, Roelfsema F, Langendonk JG, de Wit CC, Frolich M, Burggraaf J *et al*. Increased circadian prolactin release is blunted after body weight loss in obese premenopausal women. *Am J Physiol Endocrinol Metab* 2006; **290**: E218–E224.
54 Mattson MP. Hormesis defined. *Ageing Res Rev* 2008; **7**: 1–7.
55 Heilbronn LK, Civitarese AE, Bogacka I, Smith SR, Hulver M, Ravussin E. Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. *Obes Res* 2005; **13**: 574–581.
56 Lieberman HR, Caruso CM, Niro PJ, Adam GE, Kellogg MD, Nindl BC *et al*. A double-blind, placebo-controlled test of 2 d of calorie deprivation: effects on cognition, activity, sleep, and interstitial glucose concentrations. *Am J Clin Nutr* 2008; **88**: 667–676.
57 Johnstone AM, Faber P, Gibney ER, Elia M, Horgan G, Golden BE *et al*. Effect of an acute fast on energy compensation and feeding behaviour in lean men and women. *Int J Obes Relat Metab Disord* 2002; **26**: 1623–1628.
58 Varady KA, Bhutani S, Church EC, Klempel MC. Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. *Am J Clin Nutr* 2009; **90**: 1138–1143.
59 Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr* 2005; **81**: 69–73.
60 Truby H, Baic S, deLooy A, Fox KR, Livingstone MB, Logan CM *et al*. Randomised controlled trial of four commercial weight loss programmes in the UK: initial findings from the BBC 'diet trials'. *BMJ* 2006; **332**: 1309–1314.
61 Liu YM, Lacorte JM, Viguerie N, Poitou C, Pelloux V, Guy-Grand B *et al*. Adiponectin gene expression in subcutaneous adipose tissue of obese women in response to short-term very low calorie diet and refeeding. *J Clin Endocrinol Metab* 2003; **88**: 5881–5886.
62 St Pierre DH, Karelis AD, Coderre L, Malita F, Fontaine J, Mignault D *et al*. Association of acylated and nonacylated ghrelin with insulin sensitivity in overweight and obese postmenopausal women. *J Clin Endocrinol Metab* 2007; **92**: 264–269.
63 Varady KA, Roohk DJ, Loe YC, McEvoy-Hein BK, Hellerstein MK. Effects of modified alternate-day fasting regimens on adipocyte size, triglyceride metabolism, and plasma adiponectin levels in mice. *J Lipid Res* 2007; **48**: 2212–2219.
64 Kreier F, Fliers E, Voshol PJ, Van Eden CG, Havekes LM, Kalsbeek A *et al*. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat—functional implications. *J Clin Invest* 2002; **110**: 1243–1250.