Title: Compensatory mechanisms activated with intermittent energy restriction: a randomized control trial

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ABSTRACT

Background & Aims: Strong compensatory responses, with reduced resting metabolic rate (RMR), increased exercise efficiency (ExEff) and appetite, are activated when weight loss (WL) is achieved with continuous energy restriction (CER), which try to restore energy balance. Intermittent energy restriction (IER), where short spells of energy restriction are interspaced by periods of habitual energy intake, may offer some protection in minimizing those responses. We aimed to compare the effect of IER versus CER on body composition and the compensatory responses induced by WL.

Methods: 35 adults (age: 39±9 y) with obesity (BMI: 36±4 kg/m^2) were randomized to lose a similar weight with an IER (N=18) or a CER (N=17) diet over a 12 week period. Macronutrient composition and overall energy restriction (33% reduction) were similar between groups. Body weight/composition, RMR, fasting respiratory quotient (RQ), ExEff (10, 25, and 50 watts), subjective appetite ratings (hunger, fullness, desire to eat, and prospective food consumption (PFC)), and appetite-regulating hormones (active ghrelin (AG), cholecystokinin (CCK), total peptide YY (PYY), active glucagon-like peptide-1 (GLP-1), and insulin) were measured before and after WL.

Results: Changes in body weight (≈12.5% WL) and composition were similar in both groups. Fasting RQ and ExEff at 10 watts increased in both groups. Losing weight, either by IER or CER dieting, did not induce significant changes in subjective appetite ratings. RMR decreased and ExEff at 25 and 50 watts increased ($P<0.001$ for all) in IER group only. Basal and postprandial AG increased ($P<0.05$) in IER group, whereas basal active GLP-1 decreased ($P=0.033$) in CER group only. Postprandial CCK decreased in both groups ($P=0.0012$ and $P=0.009$ for IER and CER groups, respectively). No between group differences were apparent for any of the outcomes.

Conclusions: The technique used to achieve energy restriction, whether it is continuous or intermittent, does not appear to modulate the compensatory mechanisms activated by weight loss.

Keywords: Intermittent energy restriction; continuous energy restriction; body composition; energy expenditure; appetite; weight loss.

Introduction

The prevalence of obesity has increased to epidemic proportions worldwide (1). Energy restricted diets remain the major tool for obesity management which assist individuals to lose weight. Most recommendations support the use of continuous energy restriction (CER) with a consistent daily reduction in energy intake (2).

Intermittent energy restriction (IER), characterized by short spells of severe energy restriction interspaced by periods of habitual energy intake, have become a popular method of weight loss (WL) (3). This form of IER, referred to as alternate day fasting, involves a 'fast day', where food intake is either completely or partially restricted over 24 h period, alternated with a 'feed day', where food is consumed *ad libitum* over a 24 h. The fasting days vary between 2-4 days/week (4).

Few studies have compared the effects of IER with CER on body weight and composition in individuals with obesity. A recent study led by Catenacci et al. (2016) concluded that IER is a safe and tolerable approach to WL, producing similar changes in body weight and composition when compared with a CER diet (5). However, that study is limited by the fact that physical activity (PA) levels were not measured, and as a result we do not know if differential changes in PA levels in the two intervention groups could have affected the outcome variables. A review by Varady et al. (2011) which compared independent studies that were done either IER or CER diets, with no direct comparisons between the two, showed that intermittent diets are equally effective in decreasing body weight and fat mass, although IER may be more effective in minimizing the loss of FFM (4). However, in a 6 months RCT (comparing IER with CER) conducted by Harvie et al. (2011) was shown no differences between groups in the body composition changes (3). More research comparing protocols of IER with CER diets is needed, given the few available studies, methodological limitations such as lack of a comparison group and/or not controlling for PA, and conflicting results. Furthermore, what has yet to be determined is whether IER offers some protection in terms of minimizing some of the compensatory mechanisms known to be activated during WL (6-9).

Deliberate periods of energy balance during WL – as in IER – could attenuate or deactivate various adaptive responses to energy restriction, and thereby reduce the risk of weight regain (10).

The main challenge in obesity management is that WL is usually not sustained in the long-term (6, 9, 11), and the majority experience weight regain over time (12). Even though reduced motivation and compliance with the intervention are likely also to be involved in weight regain (13, 14), diet-induced WL is known to activate metabolic adaptations (11, 15), which increase the risk of relapse. These include a reduction in total energy expenditure (16), driven by a reduction in both resting and non-resting metabolic rate (16, 17). The mechanism responsible is likely to be a combination of both - an increase in exercise efficiency (ExEff) (18) plus a reduction in PA (19). Moreover, WL is also known to be associated with a reduction in fat oxidation (20), and an increase in the drive to eat (11, 21). Changes in appetite-regulating hormones favoring increased hunger and reduced fullness have been described with WL (22, 23), including an increase in the concentrations of the orexigenic hormone ghrelin (24), and a reduction in the concentrations of anorexigenic hormones such cholecystokinin (CCK), peptide YY (PYY), and glucagon-like peptide-1 (GLP-1) (25-27).

No randomized control trials to date have examined the effects of IER on the type or strength of compensatory mechanisms activated during WL. Therefore, this experimental study aimed to explore the impact of IER versus CER, inducing a similar WL, on body composition and compensatory responses (resting metabolic rate (RMR), ExEff, respiratory quotient (RQ) and appetite) in adults with obesity.

Materials and Methods

Participants

Adults (18-65 years of age, both genders) with obesity ($30<BMI<40$ kg/m^2) were recruited through advertisement posted in the local newspaper and surrounding community in Trondheim, Norway.

92 The study was approved by the local Regional Ethics Committee (Midt-Norway, Trondheim, Norway),
93 and conducted according to the guidelines laid down in the Declaration of Helsinki. All participants
94 provided written informed consent before enrolling in the study. The study was registered in
95 clinicaltrial.gov under the number NCT02169778.

96 Inclusion criteria included weight stability (no large weight fluctuations during the previous 3 months (+/-
97 2 kg)) and having a sedentary lifestyle (not engaged in strenuous work or in regular brisk leisure time
98 exercise more than once a week or in light exercise for more than 20 minutes/day in more than 3
99 times/week). Women were required to have a regular menstrual cycle (28+/-2 days). Those with clinical
100 significant illness, including diabetes, or those who had WL surgery and/or those taking medication
101 known to affect appetite or induce WL, and milk intolerance were excluded.

102

103 *Sample size estimation*

104 Twelve participants would be needed to detect a difference of 4pM x hour/L in the area under the curve
105 (AUC) for GLP-1 between the two groups, assuming a standard deviation of 2 pM x h/L, at a power of
106 80%, and a significance level of 5%. To allow for a dropout rate of 25%, a minimum of 15
107 participants/group was deemed necessary.

108

109 *Study design*

110 Participants were randomized, using simple randomization, to one of two intervention groups: (1) an IER
111 or (2) a CER diet over 12 weeks WL, with the sequence determined using a web-based randomization
112 system (WebCRF). Both interventions aimed at the same overall energy restriction (33% reduction of the
113 estimated energy needs; measured RMR x PAL (1.4)), and macronutrient composition (20% protein, 30%
114 fat, and 50% carbohydrate). Participants were asked not to change their PA levels throughout the study.

115 *Detailed protocol*

116 The IER group underwent 3 non-consecutive days of partial fasting per week. During those 3 days,
117 participants followed a commercial very low calorie diet (VLCD) (550 and 660 kcal/day for women and
118 men, respectively) (Allévo, Karo Pharma AB, Sweden), plus were allowed to have low-starch vegetables
119 (maximum 2 cups/day). For the feeding days, a diet matching energy needs was prescribed, using
120 conventional food.

121 The CER group followed a low calorie diet (LCD) using conventional food every day. In both groups, the
122 participants were encouraged to consume at least 2.5 liters of non-caloric liquids/day. For more details
123 regarding the dietary plan of both groups see tables S1, S2 and S3 in Supplementary tables.

124 Energy prescription was reviewed throughout the trial (weeks 4 and 8) to account for changes in weight
125 and RMR, in order to maintain a 33% energy restriction below estimated requirements for weight
126 maintenance.

127

128 *Compliance*

129 Diet: All participants kept daily food records and were scheduled for weekly visits for weight monitoring
130 and diet counselling with a trained dietitian. Food diaries for weeks 1, 4, 8 and 12 were analyzed for
131 nutrient content in both groups using Mat på data version 5.1 (Mattilsynet og Helsedirektoratet, Norway).

132 Physical activity: All participants were asked to use armbands (SenseWear, Body Media, Pittsburg, USA)
133 for one week, at baseline and again at weeks 6 and 12. For data to be considered valid, participants needed
134 to wear the armbands for ≥4 days, including at least 1 weekend day, and more than 95 % of data needed to
135 be available over a 24 h period. Time spent in sedentary, light, moderate and vigorous to very vigorous
136 activity, and number of steps/day were analysed.

137

Data collection

Testing was performed at baseline and after the WL intervention (week 13; at least 3 days after the end of the IER diet to eliminate the potential impact of acute partial fasting during the VLCD days).

Body weight and composition

Air displacement plethysmography (Bod Pod Life Measurement, Inc., Concord, CA, USA) was used.

RMR and fasting RQ

RMR and fasting RQ were measured by indirect calorimetry (Vmax Encore 29N, Care Fusion, Germany), using standard reference method procedures (28).

Exercise efficiency

ExEff was measured by graded cycle ergometry, immediately after the blood sampling was completed. Participants pedaled at 60 rpm against graded resistance to generate 10, 25 and 50 watts of power in successive 4 minutes' intervals. Oxygen uptake (VO2) and carbon dioxide (VCO2) production were measured continuously using a metabolic cart (Monark, Eromedic 839E, GIH, Sweden). ExEff was expressed as net efficiency (NE) (29).

Appetite measurements

In the evening before each blood sampling day, participants were asked to fast from 8pm. They were also asked to avoid exercise during the 24 hours prior to laboratory testing. A standard breakfast containing approximately 600 kcal (17 % protein, 35 % fat, and 48 % carbohydrate) was provided. Prior to eating the

standard breakfast (fasting state) and every 30 minutes after eating breakfast, for a consecutive period of 2.5 hours, subjective appetite ratings (hunger, fullness, desire to eat, and prospective food consumption (PFC)) were measured using a validated 10 cm visual analogue scale (30), and blood samples were collected (using an intravenous cannula). Plasma samples were analyzed for active ghrelin (AG), total PYY, active GLP-1, and insulin, using an Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore), and CCK, using an "in-house'' RIA method (31). The intra- and inter-assay variation was respectively <10% and <7% for AG, <5% and 15% for CCK, <20% and <4% for PYY, <20% and <7% for GLP-1, and <10% and <5% for insulin.

Statistical analysis

Data was analyzed using SPSS version 21 (SPSS Inc., Chicago, IL). Attrition was low (4 in IER group and 3 in CER group) so analysis was conducted in completers only. Results are expressed as mean ± SEM and significance level was assumed at $P<0.05$, unless otherwise stated. Data were analyzed using linear mixed-effects models (LMM), with restricted maximum-likelihood estimation of the parameters. The LMM included time and group, as well as their interaction, as fixed factors. The mean values at baseline were constrained to be equal for the two groups, due to the randomization. AUC for subjective appetite ratings and appetite hormones was calculated from 0 to 150 minutes post-prandially using the trapezoid rule.

Results

Study participants

Thirty-five subjects fulfilled all the criteria and entered the study. Participants were randomized to one of two WL groups: IER (n=18) or CER (n=17). Seven did not complete the whole protocol. One dropped out

due to pregnancy (IER group), two found it too hard to adhere to the diet (one from each group), three withdrew because of personal reasons (two from IER and one from CER group), and one was excluded due to lack of compliance (CER group) (see Figure 1). There were no significant differences between those who completed the study and those who withdraw, in terms of age or baseline BMI.

The baseline characteristics of completers are shown in Table 1. There were no significant differences between groups at baseline for any of the variables studied.

Compliance

Diet: No significant differences between groups were found in total energy intake or macronutrient composition of the diets over time (see Supplementary table S4), and energy and macronutrient distribution during the fast days of the IER was as planned (see Supplementary table S5).

Physical activity: All participants were sedentary at baseline. Steps/day increased significantly from baseline to week 6 (P=0.009), and decreased significantly from week 6 to 12 in the CER group only (P=0.017), and changes were significantly different between groups (P=0.002 and P=0.007, respectively). However, no significant differences within or between groups were seen when comparing steps/day at baseline with week 12. Moreover, no significant differences were seen in any of the other PA variables studied within or between groups over time (see Supplementary table S6).

Body weight and composition

Both groups lost a significant but similar amount of weight (≈12.5% of initial weight, P<0.001 for both groups, P=0.126 between groups), FM (in kg and %, P<0.001 for both) and FFM (in kg, P<0.001 for both), and increased their FFM (in %, P<0.001 for both) (see Table 2).

206 *RMR and fasting RQ*

207 The IER group experienced a significant reduction in RMR (P<0.001) with WL, while no significant
208 change was seen in the CER group. However, changes over time were not significantly different between
209 groups. When RMR was expressed per kg FFM, there were no longer significant changes over time in the
210 IER group, and the differences between groups remained non-significant. Fasting RQ decreased
211 significantly with WL in both groups (P=0.013 and P=0.005 for IER and CER groups, respectively), and
212 changes over time were not significantly different between groups (see Table 2).

213

214 *Exercise efficiency*

215 After WL, a significant increase in NE at 10 watts was observed in both groups (P<0.001 and P=0.010 for
216 IER and CER groups, respectively). NE at 25 and 50 watts increased significantly in the IER group only
217 (P<0.001 for both). However, differences between groups were not significant (see Table 2).

218

219 *Subjective appetite ratings*

220 Fasting and postprandial feelings of hunger, fullness, desire to eat, and PFC at baseline and at end of WL
221 phase in both groups are shown in Figure 2. At baseline there were no statistical significant differences
222 between groups. Moreover, there were no significant changes over time in any of the groups, or
223 differences between groups, for any of the subjective appetite ratings analyzed, either fasting or post-
224 prandially.

Appetite-regulating hormones

Basal and postprandial plasma concentrations of AG, CCK, total PYY, active GLP-1, and insulin at baseline and end of WL phase, in both groups are shown in Figure 3. At baseline there were no statistical significant differences between groups.

At the end of WL, the IER group had a significant increase in basal AG plasma concentrations (P=0.046), and the CER group showed a tendency towards an increase (P=0.058), without significant differences between groups. Basal active GLP-1 plasma concentrations decreased significantly in the CER group only (P=0.033), but with no significant differences between groups. Both groups experienced a significant and similar reduction in basal insulin plasma concentrations with WL (P<0.001 and P=0.042 for IER and CER groups, respectively). There were no significant changes in basal CCK or total PYY plasma concentrations over time within groups or differences between groups.

Postprandial AG increased significantly with WL in the IER group only (P=0.014), but with no significant differences between groups. After WL, both groups had a similar reduction in postprandial CCK (P=0.012 and P=0.009 for IER and CER groups, respectively), and in postprandial insulin (P<0.001 and P=0.002 for IER and CER groups, respectively). No significant changes over time within groups or differences between groups were observed for postprandial total PYY or active GLP-1.

Discussion

Reduced body weight is associated with compensatory responses, both at the level of energy expenditure and at level of the appetite control system, which appear to work in simultaneous to bring energy balance back to its original state, thus, increasing the risk of relapse (6). The original hypothesis that WL induced by an IER diet induces weaker compensatory responses, when compared with CER diet, was not confirmed in this controlled clinical study.

248 The results demonstrate a similar reduction in body weight (12.5% WL) and changes in body composition
249 (decreased FM (in kg and %) and FFM (kg), and increased FFM (%)) after 12 weeks of diet-induced WL,
250 either by IER or CER isocaloric dieting. This is consistent with the available literature. Other randomized
251 controlled trials comparing these two dietary approaches had already reported IER to be as effective as
252 CER in inducing WL (3, 32-34). Moreover, both short- (12 weeks) (33, 35) and long-term interventions (6
253 months) (3) report similar changes in body composition, regardless of the energy deficit being created by
254 an IER or CER diet, if WL is matched (3, 33, 35).

255 The percent WL experienced by our IER group (~12.5% from baseline) was greater than what has been
256 reported in prior alternate day fasting literature, which typically reports a WL of 4-7% from baseline, with
257 3-4 fast days per week (36). We believe that the differences may be related to the fact that the diets were
258 adjusted at weeks 4 and 8. In those weeks, RMR was measured again, and individual energy requirements
259 were recalculated, in order to maintain a 33% energy restriction below estimated requirements for weight
260 maintenance. Also, in our IER group, the diet on the non-fasting days was matched for the energy needs,
261 while the majority of the studies on alternate day fasting have used *ad libitum* diets during the non-fasting
262 days, which might have reduced the overall energy deficit of the diet.

263 Varady et al. in their review (2011) showed more favorable changes in body composition when WL was
264 induced by an IER diet versus CER diet. They compared the effects of IER (5 studies) with CER (11
265 studies) on body weight/composition, in individuals with overweight/obesity. They reported an overall
266 WL distributed as 90% FM and 10% FFM in the IER trials versus 75% FM and 25% FFM in the CER
267 trials (4), suggesting that IER is better in minimizing the loss of FFM observed with WL. The reviewed
268 studies were all RCTs, and matched for WL (%). However, different methods were used for measuring
269 body composition. In some of the studies body composition was measured by BIA (in the majority of IER
270 trials), a method known to overestimate FM (%) in people with obesity (37), while the majority of CER
271 trials used dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging, more accurate
272 techniques for the assessment of FM and FFM (38).

In the present study, a significant reduction in RMR was observed in the IER group only, without significant differences between groups. However, after adjusting for FFM (in kg), which is the main determinant of RMR (16, 39, 40), there were no longer significant changes in RMR in the IER diet group, and the differences between groups over time remained non-significant. Not many studies have measured RMR in response to IER. Arguin et al. (2012) reported no changes in RMR and no differences between the IER and CER groups after 12% WL, despite a better preservation of FFM in the last group, in a RCT study with 25 women with obesity (32). Similar results were found by Heilbronn et al. (2005), where 16 lean individuals who fasted every other day for 22 days experienced no reduction in RMR, despite a WL of 2.5% (41). Catenacci et al. (2016) showed that RMR decreased significantly and similarly after 8 weeks of IER (zero-calorie alternate-day fasting) and CER (-400 kcal/day) in 25 adults with obesity (5). Interestingly, when adjusted for FM and FFM, RMR decreased significantly from baseline to week 8 in the CER group only, with a trend (P=0.076) for differences between groups. However, these results should be interpreted with caution as PA levels were not measured during the intervention.

Fasting RQ decreased significantly with WL, regardless of the nature of the energy restriction in this study. This is indicative of increased fat oxidation, probably as a result of increased fat catabolism during active WL (16), and is consistent with previous research. Heilbronn et al. (2005) reported a fasting RQ reduction in response to an IER diet over 22 days, and found that immediately after the intervention RQ was significantly reduced (41). Also in the RCT conducted by Harvie et al. (2011), an increase in fat oxidation (reduction in fasting RQ) at the end of WL intervention (7% WL) was reported, without significant differences between groups (IER versus CER) (3).

The present study is the first to investigate changes in ExEff in response to WL induced by IER. A significant increase in ExEff at 10 watts was seen in both groups after WL, whereas at 25 and 50 watts a significant increase was observed in the IER group only, but without significant differences between groups. Several studies have already described an increase in ExEff with WL induced by CER (18, 42, 43). After 10% WL, studies have shown that skeletal muscle work efficiency is increased (18, 42), and

298 this is particularly evident at lower intensity levels (10 and 25 watts) (18, 44, 45). A greater ExEff may be
299 a disadvantage in obesity management, given that less energy is required to perform the same volume of
300 exercise (45).

301 Overall, studies looking at the impact of WL (10-14%) induced by CER on subjective appetite ratings in
302 individuals with obesity tend to report an increase in hunger, desire to eat and in PFC, while fullness
303 feelings in the postprandial state are usually reduced (46, 47). Few studies have looked at changes in
304 subjective feelings of appetite after WL induced by IER (41, 48-51). In this study no significant changes
305 in subjective feelings of appetite were seen with WL and no differences between groups were found,
306 which it surprising given the magnitude of WL (12.5% WL).

307 Varady et al. (2013) reported no changes in feelings of hunger, but an increase in feelings of fullness in
308 normal-weight and overweight individuals, after 12 weeks of IER diet (75% energy restriction, 3
309 days/week, and ad libitum eating 4 days/week) inducing a 6.5% WL (48). Similar results were described
310 by Heillbronn et al. (2005) after 3 weeks of alternative day fasting inducing a 2.5% WL in normal-weight
311 individuals (41). On the other hand, Klempel et al. (2010) reported a decline in hunger after 8 weeks IER
312 (6% WL) and no change in feelings of fullness in individuals with obesity (49). Similarly, Bhutani et al.
313 (2013) reported a reduction in hunger and increase in fullness after 12 weeks of IER diet (5% WL) in
314 individuals with obesity (50). However, these findings are limited in that subjective ratings of appetite
315 were measured on the evening immediately before bed time (49, 50) and, therefore, cannot be directly
316 related to our findings. In a non-RCT study, where 59 individuals with obesity underwent 8 weeks of IER
317 (every other day 25% of baseline energy needs, other days ad libitum), postprandial hunger did not change
318 after 4% WL, but postprandial fullness increased (51). However, this study is flawed given the lack of a
319 control group.

320 In this study, a significant increase in basal AG was seen in the IER (and a trend in the CER group
321 (P=0.058)), while postprandial AG increased in the IER group only (despite no significant differences
322 between groups). Similar results were found by Catenacci et al. (2016), with a significant increase in

ghrelin plasma concentrations in IER but not in CER group, even though differences between groups were not significant (5). Regarding satiety hormones, a decrease was observed in basal active GLP-1 in the CER group only (without differences between groups), and a decrease in postprandial CCK in both groups. Most of the previous studies looking at the impact of WL induced by CER on appetite related hormones have reported a significant increase in AG and a reduction in the concentrations of satiety hormones (8, 9, 52).

It is remarkable that no changes in subjective feelings of appetite were found in the present study, when significant changes in the plasma concentrations of several appetite related hormones were reported. Similar findings were reported by Hoddy et al. (2016), with a significant increase in postprandial total ghrelin plasma concentrations, despite no change in postprandial hunger feelings after 8 weeks of IER diet (51). This is in contrast with the majority of the evidence from CER studies, which tend to show increases in both hunger and ghrelin after WL (24, 47, 53).It is important to remember that the appetite control system is extremely complex and several hormones have been shown to be involved. Hunger and fullness feelings are not the result of the changes in one single peptide (52). Even though some studies show that changes in the plasma concentrations of some appetite-related hormones are correlated with changes in some subjective feelings of appetite (54), others show no correlation (55, 56).

Moreover, although the majority of the evidence tends to show that diet-induced WL (by CER) is usually associated with an increase in ghrelin and a reduction in the concentrations of satiety hormones, a recently published study seems to challenge that. Iepsen et al. (2016) showed that a 13% WL, achieved with a very low calorie diet (VLCD), in individuals with obesity leads to a significant increase in postprandial concentrations of total ghrelin, PYY_{3-36} and total GLP-1 (57). These results are not in line with those reported by Sumithran et al. (2011), where an increase in postprandial concentrations of active ghrelin, but a decrease in total PYY and CCK, and no change in active GLP-1 were reported after a 14% WL, achieved also with a VLCD (47). These contradictory findings suggest that the adaptations to the weight

reduced state experienced at the level of the appetite control system are complex and remain to be fully understood.

The present study has both strengths and limitations. The main strength of this study is its design, randomized clinical trial. Second, the daily energy needs were estimated from measured RMR and thus individualized. Third, reference standard methods were used to measure all outcome variables. Fourth, compliance to the intervention was measured throughout the study, which was very good. Total energy and macronutrient composition of the diets were similar between groups, and PA levels did not change over time and were not different between groups. The fact that both groups lost a similar amount of weight, suggests that differences between groups are likely to be due to the diet intervention in itself. As a limitation, the participants from the CER group increased significantly their amount of steps/day from baseline to week 6, which could have impacted on the results. However, there were no significant differences between groups in steps/day during the whole intervention time. Finally, as with many weight loss studies, the majority of the participants were women, which makes it difficult to generalize the results to the whole population.

The fact that both diets were associated with similar changes in body composition and activation of compensatory mechanisms is consistent with a recently published systematic review showing no differences in long-term (>6 months) relapse between the two dietary approaches (58).

In conclusion IER seems to be as effective as CER with regards to WL, and is associated with similar changes in body composition and compensatory responses activated with weight reduction. For that reason, IER can be considered as an alternative to daily CER for individuals with obesity who wants to lose weight. However, more and larger studies, ideally with a longer follow up period, are needed.

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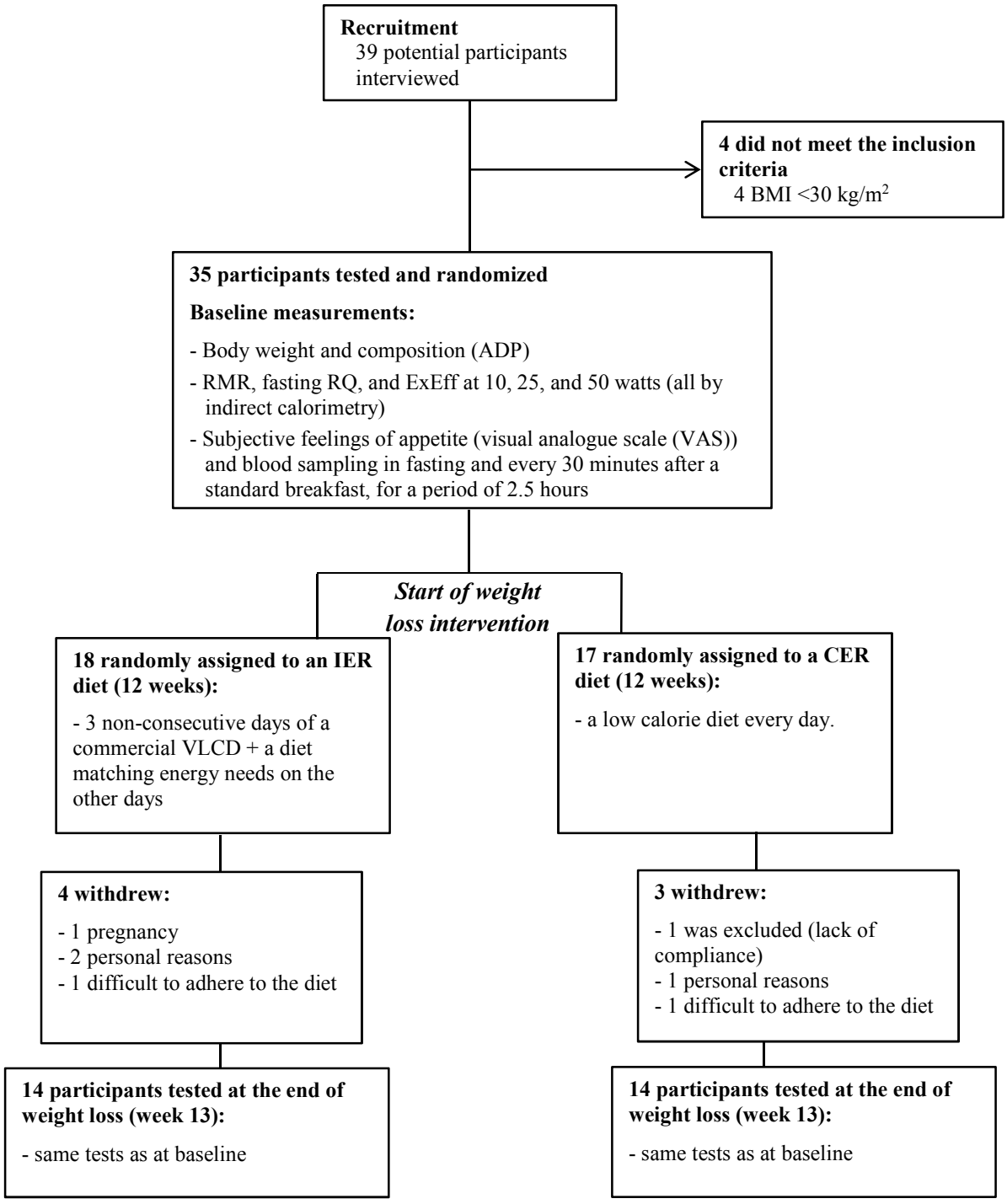
Figure 1. Study diagram



BMI: body-mass index; ADP: air displacement plethysmography; RMR: resting metabolic rate; RQ: respiratory quotient; ExEff: exercise efficiency; IER: intermittent energy restriction; CER: continuous energy restriction; VLCD: very low calorie diet.

Table 1. Baseline characteristics of the participants who completed the study

	IER group (n=14)	**CER group** (n=14)	**P-*value***
Age (years)	39.4±11.0	39.1±9.0	0.635
Gender ratio (women : men)	10 : 4	12 : 2	0.376
Body weight (kg)	107.2±13.6	97.5±12.8	0.063
BMI (kg/m^2)	35.6±3.2	35.1±4.2	0.603
Fat Mass (kg)	47.0±7.9	43.0±8.1	0.182
Fat Mass (%)	44.0±6.2	44.1±5.4	0.950
Fat Free Mass (kg)	60.4±11.8	54.5±9.1	0.141
Fat Free Mass (%)	56.1±6.2	55.9±5.4	0.943
RMR (kcal/day)	1488.1±269.6	1342.1±140.1	0.071

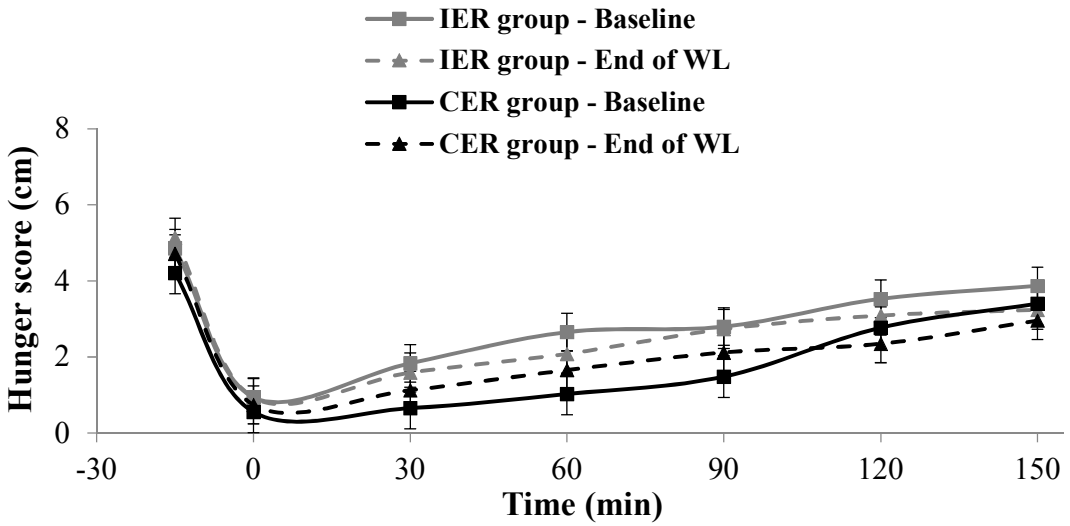
Data presented as mean ± SD. IER: intermittent energy restriction; CER: continuous energy restriction; BMI: body-mass index; RMR: resting metabolic rate. P-values for comparison between groups at baseline.

Table 2. Changes in anthropometric measurements, RMR, fasting RQ, and exercise efficiency in the IER and CER groups

	IER group			**CER group**			**P-*value*****
	Baseline	**End of WL**	**P-*value****	**Baseline**	**End of WL**	**P-*value****	
Weight (kg**)**	107.2±3.4	93.3±3.4	<0.001	97.5±3.4	85.7±3.4	<0.001	0.089
FM (kg**)**	47.0±2.0	35.7±2.0	<0.001	43.0±2.0	33.4±2.0	<0.001	0.141
FM (%**)**	43.9±1.6	38.5±1.6	<0.001	44.1±1.6	38.9±1.6	<0.001	0.706
FFM (kg**)**	60.4±2.7	57.6±2.7	<0.001	54.5±2.7	52.6±2.7	<0.001	0.262
FFM (%**)**	56.1±1.6	61.5±1.6	<0.001	55.9±1.6	61.1±1.6	<0.001	0.741
RMR (kcal/day**)**	1488±55	1368±55	<0.001	1342±55	1302±55	0.193	0.151
RMR_{FFM} (kcal/day/kg FFM**)**	24.7±0.55	23.9±0.55	0.114	24.9±0.55	25.0±0.55	0.822	0.119
Fasting RQ	0.86±0.01	0.81±0.01	0.013	0.87±0.01	0.82±0.01	0.005	0.908
NE (10 W**)**	0.051±0.003	0.065±0.003	<0.001	0.055±0.003	0.062±0.003	0.010	0.069
NE (25 W**)**	0.102±0.005	0.124±0.005	<0.001	0.113±0.005	0.118±0.005	0.274	0.065
NE (50 W**)**	0.148±0.006	0.175±0.006	0.001	0.158±0.005	0.165±0.005	0.262	0.098

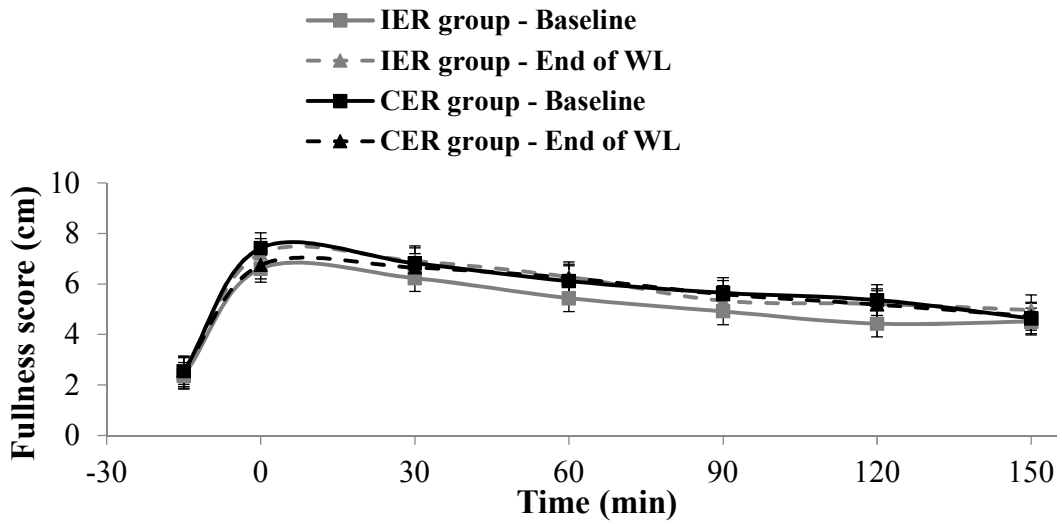
Data presented as mean ± SEM. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss; FM: fat mass; FFM: fat free mass; RMR: resting metabolic rate; RQ: respiratory quotient; NE: net efficiency. *P-values are for changes between time points within groups. **P-values are for comparisons between groups for changes over time. Data were analyzed using linear mixed-effect models (LMM), with restricted maximum-likelihood estimation. The LMM included time, group, and their interaction as well as fixed factors.

Figure 2a. Fasting and postprandial ratings of hunger over time in both groups



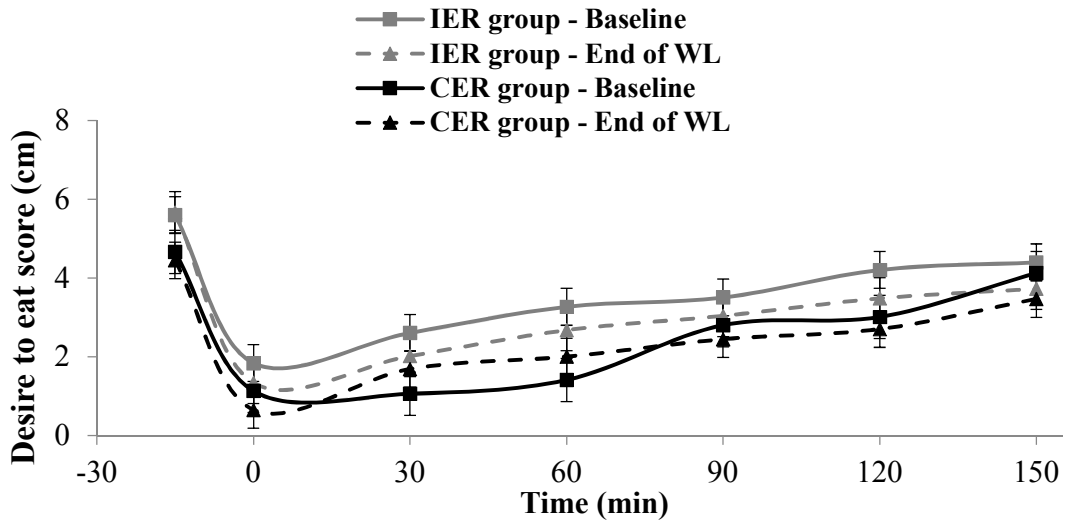
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 2b. Fasting and postprandial ratings of fullness over time in both groups



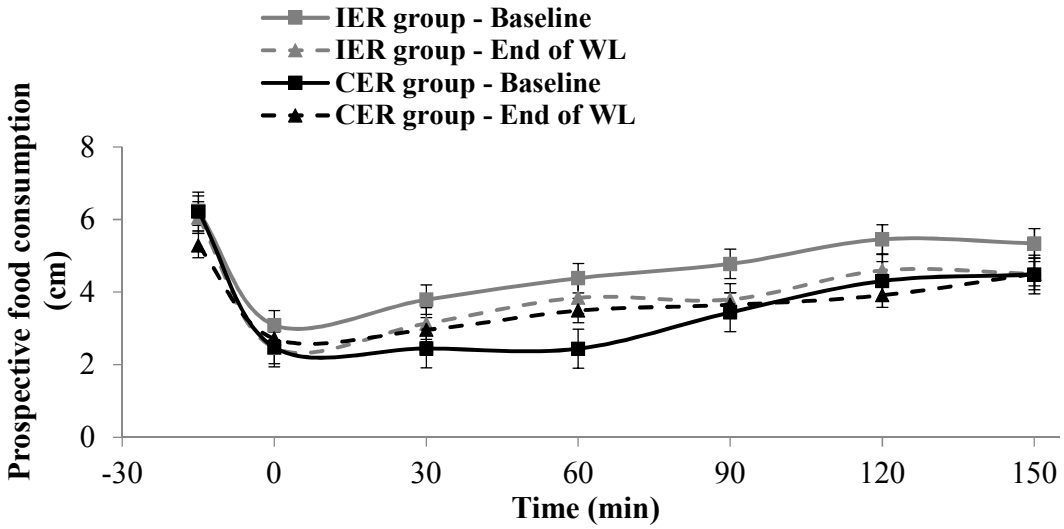
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 2c. Fasting and postprandial ratings of desire to eat over time in both groups



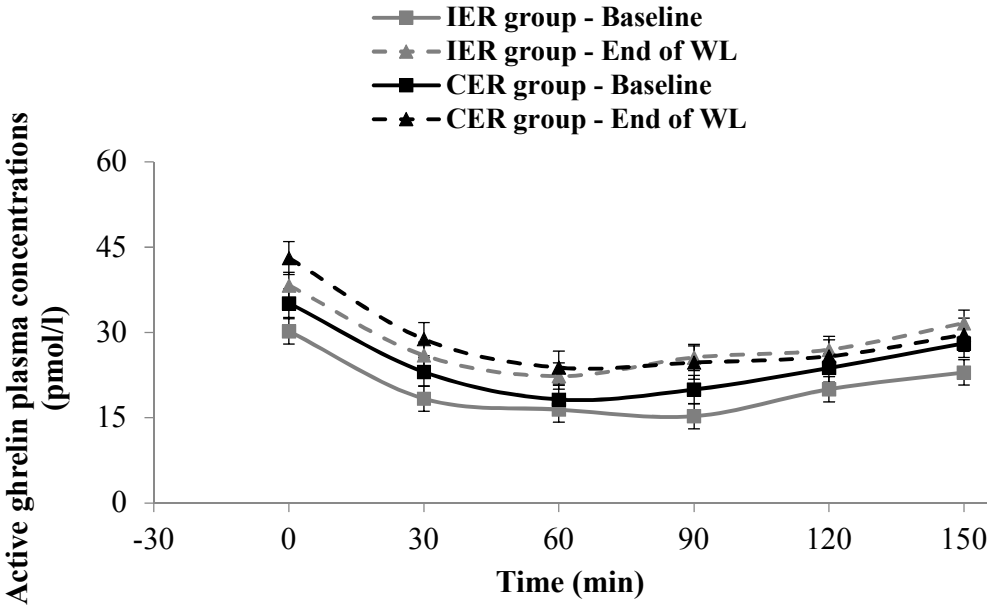
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 2d. Fasting and postprandial ratings of prospective food consumption over time in both groups



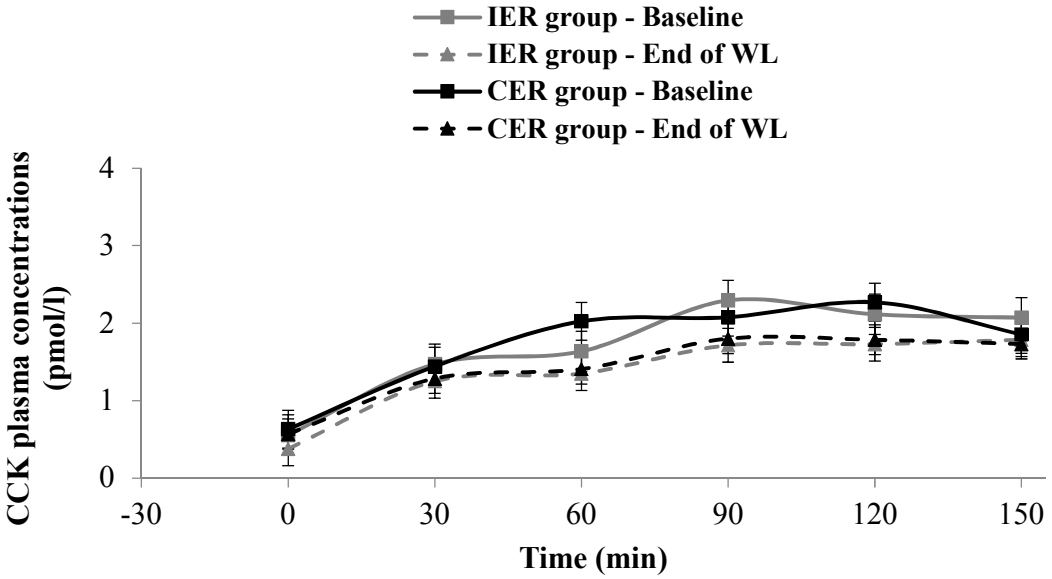
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 3a. Basal and postprandial plasma concentrations of active ghrelin over time in both groups



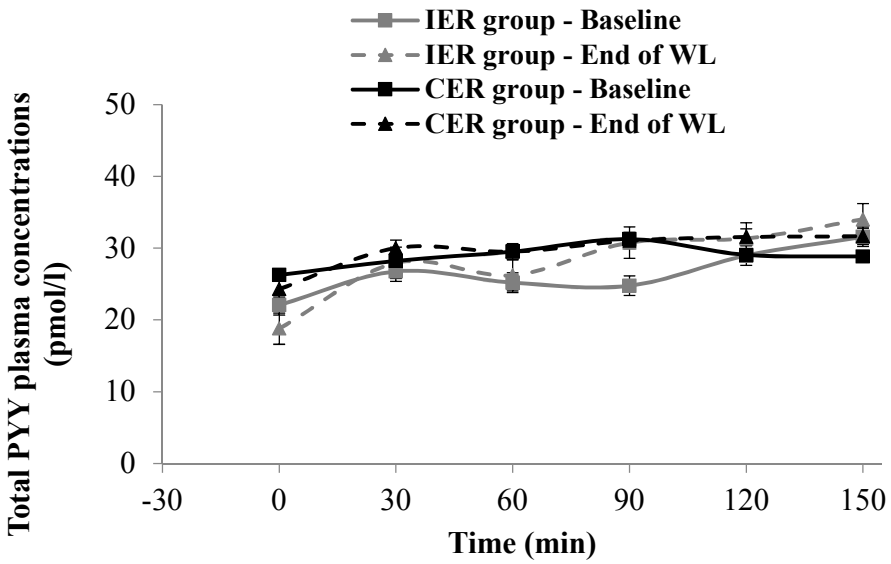
Plasma concentrations (pmol/l) of active ghrelin over time (min) in both groups, fasting and after intake of a standard breakfast. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 3b. Basal and postprandial plasma concentrations of CCK over time in both groups



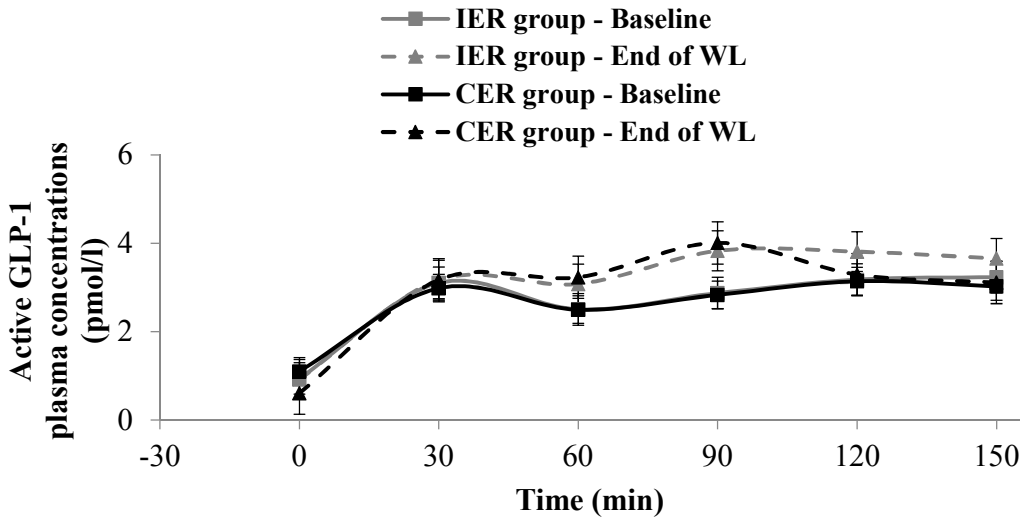
Plasma concentrations (pmol/l) of CCK over time (min) in both groups, fasting and after intake of a standard breakfast. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 3c. Basal and postprandial plasma concentrations of total PYY over time in both groups



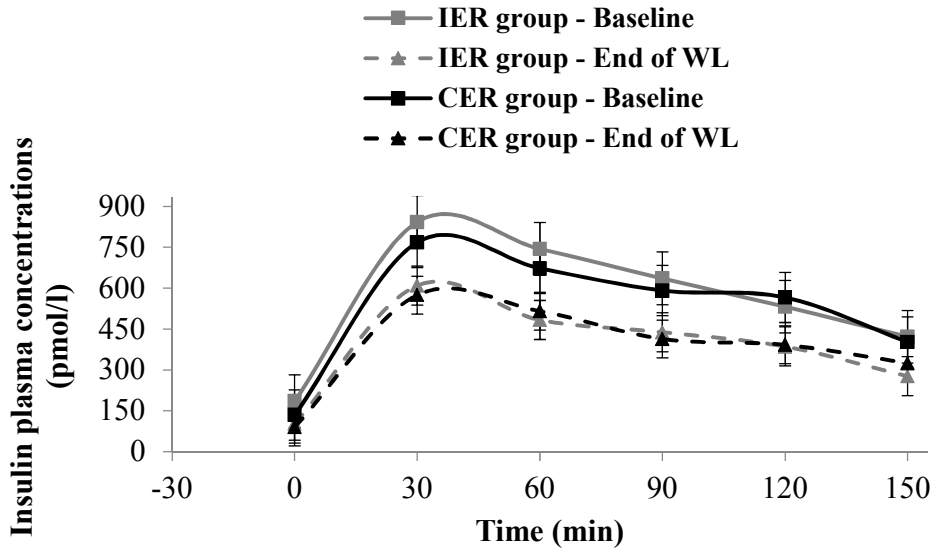
Plasma concentrations (pmol/l) of total PYY over time (min) in both groups, fasting and after intake of a standard breakfast. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 3d. Basal and postprandial plasma concentrations of active GLP-1 over time in both groups



Plasma concentrations (pmol/l) of active GLP-1 over time (min) in both groups, fasting and after intake of a standard breakfast. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Figure 3e. Basal and postprandial plasma concentrations of insulin over time in both groups



Plasma concentrations (pmol/l) of insulin over time (min) in both groups, fasting and after intake of a standard breakfast. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data presented as mean (± SEM) at baseline, and end of the WL phase.

Table S1. Dietary plan for the IER group in the fasting (VLCD) days

Meal	**Women (**550 kcal/day**)**	**Men (**660 kcal/day**)**
Breakfast	1 shake	1 shake
Lunch	1 soup	1 soup
Snack	1 shake	1 shake
Dinner	1 soup + Max. 50 g of low-starch vegetables	1 soup + 1 shake + Max. 50 g of low-starch vegetables
Snack	1 shake	1 shake

IER: intermittent energy restriction; VLCD: very low calorie diet.

Table S2. Example of a dietary plan for IER group in the feeding days (≈2118 kcal)

Meal	**Food description**
Breakfast	2 slices wholegrain bread (40 g per bread) **or** 4 whole grain toasts (13 g per toast) 1 slice ham / other lean meat **or** 15 g of lean pate 2 tbsp. jam (16 g per tablespoon) 5 g butter 20 g paprika **or** 30 g tomato **or** 50 g cucumber 1 glass of low fat milk (1.5 dl) 1 apple **or** pear **or** peach **or** orange (130-180 g per fruit)
Snack	1 large banana **or** 40 g raisins / other dried fruit
Lunch	4 whole grain toasts (13 g per toast) **or** 2 wholegrain bread ½ avocado (50 g) **or** 25 g light mayonnaise 2 slices ham / other lean meat **or** 30 g of lean pate 1 tbsp. jam (16 g per tablespoon) 10 g low fat cheese (9 %) 1 carrot **or** turnip (60-70 g)
Snack	1 slice wholegrain bread (40 g per bread) **or** 2 whole grain toasts (13 g per toast) 2 tbsp. jam (16 g per tablespoon) 1 apple **or** pear **or** peach **or** orange (130-180 g per fruit)
Dinner	100 g poultry, meat **or** fish 3 tbsp. oil (olive or canola oil) for cooking 150 g of cooked rice **or** pasta, macaroni, spaghetti **or** 3 small potatoes (250 g) **or** 60 g tortilla 200 g cauliflower / broccoli / tomato / cucumber 1 apple **or** pear **or** peach **or** orange (130-180 g per fruit)
Snack	2 slices wholegrain bread (40 g per bread) **or** 4 whole grain toasts (13 g per toast) 2 tbsp. jam (16 g per tablespoon) 10 g butter

IER: intermittent energy restriction.

Table S3. Example of a dietary plan for CER group (≈1410 kcal)

Meal	Food description
Breakfast	2 slices wholegrain bread (40 g per bread) **or** 4 whole grain toasts (13 g per toast) **or** 1 cup (1 dl) oatmeal 2 slices ham / other lean meat **or** 30 g of lean pate 20 g paprika **or** 30 g tomato **or** 50 g cucumber 1 glass of low fat milk (1.5 dl) 1 apple **or** pear **or** peach **or** orange (130-180 g per fruit)
Snack	1 large banana **or** 30 g raisins / other dried fruit
Lunch	4 whole grain toasts (13 g per toast) **or** 2 wholegrain bread ½ avocado (50 g) **or** 25 g light mayonnaise 1 slice ham / other lean meat **or** 15 g of lean pate 10 g low fat cheese (9 %) 1 carrot **or** turnip (60-70 g)
Snack	1 slice wholegrain bread (40 g per bread) **or** 2 whole grain toasts (13 g per toast) 1 tbsp. jam (16 g per tablespoon)
Dinner	100 g poultry, meat **or** fish 15 g oil (olive or canola oil) for cooking 100 g of cooked rice **or** pasta, macaroni, spaghetti **or** 2 small potatoes (170 g) **or** 40 g tortilla 200 g cauliflower / broccoli / tomato / cucumber
Snack	1 slice wholegrain bread (40 g per bread) **or** 2 whole grain toasts (13 g per toast) 1 tbsp. jam (16 g per tablespoon) 5 g butter

CER: continuous energy restriction.

Table S4. Daily energy intake and macronutrient composition of the diets in the IER and CER groups at weeks 1, 4, 8, and 12

	IER group				**CER group**			
	1st week	**4th week**	**8th week**	**12th week**	**1st week**	**4th week**	**8th week**	**12th week**
Energy (kcal/day**)**	1500±58.6[a]	1511±58.6[b]	1454±58.6	1382±58.6[a,b]	1334±58.6	1364±58.6[a]	1301±58.6	1255±58.6[a]
Protein (g**)**	75.4±2.8[a]	74.9±2.8[b]	71.0±2.8	67.9±2.8[a,b]	62.7±2.8	64.5±2.8	60.0±2.8	60.2±2.8
Protein (%**)**	20.2±0.4	20.0±0.4	19.6±0.4	19.7±0.4	18.8±0.4	19.0±0.4	18.5±0.4	19.3±0.4
Fat (g**)**	44.0±2.0	46.0±2.0	43.4±2.0	42.3±2.0	38.0±2.0	38.7±2.0	36.9±2.0	36.4±2.0
Fat (%**)**	26.6±0.7	27.3±0.7	26.9±0.7	27.5±0.7	25.7±0.7	25.4±0.7	25.5±0.7	26.1±0.7
CHO (g**)**	184.2±8.1[a]	182.6±8.1	178.1±8.1	167.6±8.1[a]	169.5±8.1	173.5±8.1	166.8±8.1	158.9±8.1
CHO (%**)**	48.8±0.7	48.2±0.7	49.0±0.7	48.4±0.7	50.8±0.7	51.0±0.7	51.3±0.7	50.6±0.7
Fiber(g**)**	31.3±1.9	30.8±1.9	30.7±1.9	29.1±1.9	28.6±1.9[a]	30.4±1.9[b]	28.6±1.9[c]	24.3±1.9[a,b,c]
Fiber (%**)**	4.2±0.2	4.1±0.2	4.2±0.2	4.2±0.2	4.2±0.2	4.4±0.2[a]	4.4±0.2	3.8±0.2[a]

Data presented as mean ± SEM. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss; CHO: carbohydrates. Data were analyzed using linear mixed-effect models, and Bonferroni correction was used for post hoc pairwise comparisons. Means with the same superscript letters denote significant differences between time points within groups. Significance level was assumed at $P<0.008$.

Table S5. Daily energy intake and macronutrient composition on the fast days in the IER group at weeks 1, 4, 8, and 12

	IER group			
	1st week	**4th week**	**8th week**	**12th week**
Energy (kcal/day)	590.0±23.8	593.1±23.8	591.6±23.8	592.4±23.8
Protein (g)	57.6±2.7	57.1±2.7	57.4±2.7	57.2±2.7
Protein (%)	39.1±0.3	38.5±0.3	38.8±0.3	38.6±0.3
Fat (g)	9.9±0.9	10.0±0.9	10.0±0.9	10.0±0.9
Fat (%)	15.1±0.4	15.1±0.4	15.1±0.4	15.1±0.4
CHO (g)	59.0±2.1	57.8±2.1	58.4±2.1	58.1±2.1
CHO (%)	40.0±0.3	39.0±0.3	39.5±0.3	39.2±0.3
Fiber(g)	17.2±0.7	17.9±0.7	17.6±0.7	17.7±0.7
Fiber (%)	5.8±0.1	6.0±0.1	5.9±0.1	6.0±0.1

Data presented as mean ± SEM. IER: intermittent energy restriction; CHO: carbohydrates. Data were analyzed using linear mixed-effect models, with restricted maximum-likelihood estimation, including fixed effect for time. Bonferroni correction was used for post hoc pairwise comparisons. No significant main effect of time was found. Significance level was assumed at $P<0.008$.

Table S6. Physical activity levels in the IER and CER groups at baseline, week 6 and week 12

	IER group			CER group		
	Baseline	**6th week**	**12th week**	**Baseline**	**6th week**	**12th week**
Sedentary (min**)**	1274.2±48.5	1315.9±48.5	1308.3±44.6	1287.6±43.0	1265.4±46.4	1206.8±43.0
Light (min**)**	87.8±46.3	59.7±46.3	66.3±42.6	84.4±41.1	81.6±44.3	168.3±41.1
Moderate (min**)**	52.2±9.3	37.2±9.3	44.7±8.9	49.0±8.6	54.7±8.9	43.2±8.6
Vigorous & Very vigorous (min**)**	0.4±0.5	0.4±0.5	0.5±0.5	0.7±0.4	1.8±0.5	1.3±0.4
Steps/day	7239±793.4	6218±793.4[1]	6866±769.3[2]	6716±741.3[a]	8441±762.5[a,b;1]	6854±741.3[b;2]

Data presented as mean ± SEM. IER: intermittent energy restriction; CER: continuous energy restriction; WL: weight loss. Data were analyzed using linear mixed-effect models, and Bonferroni correction was used for post hoc pairwise comparisons.
Means with the same superscript letters denote significant differences between time points within groups: [a] $P<0.01$, [b] $P<0.05$.
[1] $P=0.002$, significant differences between groups in the changes from baseline to week 6.
[2] $P=0.007$, significant differences between groups in the changes from week 6 to week 12.