Title: The impact of rate of weight loss on body composition and compensatory mechanisms during weight reduction: a randomized control trial

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Clinical Trial Registration number: NCT01912742 (the study was registered in clinicaltrial.gov).

Abstract

Background & Aims: Rapid weight loss (WL) has been associated with a larger loss of fat free mass and a disproportional reduction in resting metabolic rate (RMR), but the evidence is inconclusive. We aimed to evaluate the impact of WL rate on body composition and compensatory mechanisms activated with WL (reduced RMR, increased exercise efficiency (ExEff) and appetite), both during negative and neutral energy balance (EB).

Methods: Thirty-five participants with obesity were randomized to lose a similar weight rapidly (4 weeks) or gradually (8 weeks), and afterwards to maintain it (4 weeks). Body weight and composition, RMR, ExEff (10, 25 and 50 watts), appetite feelings and appetite-regulating hormones (active ghrelin, cholecystokinin, total peptide YY (PYY), active glucagon-like peptide-1 and insulin), in fasting and every 30 minutes up to 2.5 hours, were measured at baseline and after each phase.

Results: Changes in body weight (≈9%) and composition were similar in both groups. With WL, RMR decreased and ExEff at 10 watts increased significantly in the rapid WL group only. However, fasting hunger increased significantly with gradual WL only, while fasting and postprandial prospective food consumption, and postprandial hunger decreased (and postprandial fullness increased) significantly with rapid WL only. Basal total PYY, and basal and postprandial insulin decreased significantly, and similarly in both groups. After weight stabilization and no ketosis no differences between groups were found.

Conclusions: Despite differences while under negative EB, WL rate does not seem to have a significant impact on body composition or on compensatory mechanisms, once EB is reestablished.

Keywords: Rate of weight loss; appetite; energy expenditure; weight loss; weight maintenance.

Introduction

The worldwide prevalence of obesity has nearly doubled between 1980 and 2008 (1). Although significant weight loss (WL) can be achieved by a combination of dietary restriction and increased physical activity (PA) (2), only approximately 15% of individuals with obesity succeed in maintaining WL in the long-term (3).

The problem of weight relapse is likely to be due, among other factors, to the activation of compensatory metabolic responses triggered by WL (4, 5). These include a reduction in both resting and non-resting energy expenditure (6). The latter is thought to be driven mainly by an increase in exercise efficiency (ExEff) (7). Moreover, an increase in the drive to eat has also been reported with WL (5). The increased hunger and decreased fullness observed after WL are driven, at least partially, by changes in the plasma concentrations of appetite-regulating hormones (8). It has been demonstrated that concentrations of anorexigenic hormones such as cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), and insulin decrease with WL, whereas the concentration of the orexigenic hormone ghrelin increase (9, 10).

International guidelines regarding obesity management recommend gradual WL (0.5-1 kg/week) (2). There is a common belief that losing weight rapidly is associated with poorer outcomes, namely a greater loss of fat free mass (FFM) (11), a reduction in RMR greater than predicted (12), and more weight regain in the long-term (13). However, these assumptions suffer from several methodological limitations, such as lack of randomization and/or not controlling for magnitude of WL (14, 15). In fact, the potential advantage of losing weight gradually has been recently questioned (16), and losing weight fast, with very low calorie diets (VLCD), has been associated with better (17) or similar (18, 19) WL maintenance in the long-term. Moreover, no studies have evaluated if WL rate has an impact on the strength of compensatory mechanisms activated with WL, so more studies are required.

This study aimed to explore the impact of WL rate (rapid *vs* gradual) on body composition and compensatory mechanisms (RMR, ExEff and appetite).

Materials and Methods

Participants

Adults (18-65 years old) with obesity ($30 < \text{BMI} < 45$ kg/m^2) were recruited. The study was approved by the local Regional Ethics Committee (Midt-Norway, Trondheim, Norway). All participants provided written informed consent before enrolling in the study. The study was registered in clinicaltrial.gov (NCT01912742).

Participants had to be weight stable over the past 3 months (+/- 2 kg) and have a sedentary lifestyle. Women were required to have a regular menstrual cycle (28+/-2 days). Persons with clinical significant illness, including diabetes, with previous WL surgery and/or those taking medication known to affect appetite or induce WL were excluded.

Sample size estimation

Sixteen participants would be needed to detect a difference of 6.5 pM x hour/L in the area under the curve (AUC) for GLP-1 between the two groups, assuming a standard deviation of 6.2 pM x h/L, at a power of 80%, and a significance level of 5%.

Study Design

Participants were randomized to one of two intervention groups: (1) rapid or (2) gradual WL with the sequence determined using a web-based randomization system (WebCRF). Allocation concealment was enforced. Both interventions were designed to achieve a similar WL (9-10% WL). Participants were asked not to change their PA levels throughout the study.

Detailed Protocol

- Weight loss phase

The rapid WL group was provided with a commercial VLCD (550 and 660 kcal/day for women and men, respectively) (Allévo, Karo Pharma AB, Sweden) for 4 weeks.

The gradual WL group was provided with a low calorie diet (LCD) (1200 and 1500 kcal/day for women and men, respectively) using meal replacements (Allévo, Karo Pharma AB, Sweden) plus conventional foods for 8 weeks (see Tables S1 and S2 in Supplementary Tables). The macronutrient composition of the diets was matched (% energy provided by each macronutrient): 38.9% protein, 16.4% fat, 40.0% carbohydrates (CHO), 5.9% fiber (VLCD 550 kcal/day: 54 g protein, 10 g fat, 55 g CHO, 16 g fiber; VLCD 660 kcal/day: 64 g protein, 12 g fat, 66 g CHO, 20 g fiber, and LCD 1200 kcal/day: 117 g protein, 22 g fat, 120 g CHO, 35 g fiber; LCD 1500 kcal/day: 146 g protein, 27 g fat, 150 g CHO, 44 g fiber).

- Weight loss maintenance phase

After WL, participants were prescribed an individual diet plan by a dietitian based on their energy requirements (measured, RMR x PAL (1.4)), with a macronutrient composition of 15-20% protein, 20-30% fat, 50-60% CHO, aiming at weight stabilization for 1 month.

Compliance

Diet: All participants kept daily food records and were monitored weekly by a dietitian. Food diaries were analyzed using Mat på data version 5.1 (Mattilsynet og Helsedirektoratet, Norway). Urine acetoacetic acid concentration was measured weekly, using Ketostix reagent strips, as a measure of compliance in the rapid WL group.

Physical Activity: SenseWear (Body Media, Pittsburg, USA) devices were worn for one week, at baseline, weeks 2+4 and 4+8 for the rapid and gradual WL groups, respectively, and again (for both groups) at the last week of the WL maintenance. Data was considered valid if participants wear the armbands for ≥4 days, including at least 1 weekend day, on more than 95% of the time (20).

Data collection

Testing was performed at baseline, after WL (weeks 5 and 9 for rapid and gradual WL groups, respectively), and after WL maintenance (weeks 9 and 13 for rapid and gradual WL groups, respectively).

Body weight and composition

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

RMR

RMR was measured using indirect calorimetry (Vmax Encore 29, Care Fusion, Germany) using standard reference method procedures (21).

Exercise efficiency

ExEff was measured by graded cycle ergometry, immediately after blood sampling. Participants pedaled at 60 rpm against graded resistance to generate 10, 25 and 50 watts of power in successive 4 minutes intervals. Gas exchange was measured continuously using a metabolic cart (Monark, Eromedic 839E, GIH, Sweden). ExEff was expressed as net efficiency (NE) (7).

Appetite measurements

In fasting and every 30 minutes after a standard breakfast, for a period of 2.5 hours, appetite feelings (hunger, fullness, desire to eat, and prospective food consumption (PFC)) were measured using a validated 10 cm visual analogue scale (22), and blood samples were collected. A standard breakfast containing 600 kcal (17% protein, 35% fat, 48% CHO) was consumed within 20 minutes. 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 (23).

Statistical analysis

Data was analyzed using SPSS version 21 (SPSS Inc., Chicago, IL). Attrition was low (1 per 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. Bonferroni correction was used for post hoc pairwise comparisons for the fixed effects, with a significant level $P<0.017$. AUC for appetite feelings and appetite hormones was calculated using the trapezoid rule from 0 to 150 minutes postprandially.

Results

Participants

Thirty-five subjects entered the study with 18 randomized to the rapid and 17 to the gradual WL group (see Figure 1). Baseline characteristics are shown in Table 1. There were no significant differences between groups at baseline for any of the variables studied.

Compliance

Diet: Significant differences between groups were found in total energy intake and macronutrient composition (% and g) of the diets in the last week of the WL phase. The % energy provided by fat and CHO was significantly lower in the rapid WL group (fat, $P<0.001$ and CHO, $P<0.001$), and the % energy provided by protein and fiber was significantly higher in the rapid WL group (protein, $P=0.001$ and fiber, $P<0.001$). Regarding absolute amount of macronutrients (g/day), intake of protein, fat, CHO and fiber was significantly higher in the gradual WL group ($P<0.001$) (see Supplementary Table S3).

The mean acetoacetic acid concentration in the urine, during the WL phase in the rapid WL group, was 2.1±2.9 mmol/L.

Physical activity: All participants were sedentary and there were no significant changes over time in any PA variable studied within or between groups (see Supplementary Table S4).

Body weight and composition

Both groups lost a significant and similar amount of weight (≈9%, $P<0.001$ for both groups, $P=0.160$ between groups), fat mass (FM) (kg and %, $P<0.001$ for both) and FFM (kg, $P<0.001$ for both), and increased their FFM (%, $P<0.001$ for both) with WL, with no significant differences between groups. The rapid WL group lost weight at a rate of 2.2±0.1 kg/week and the gradual at a rate of 1.2±0.1 kg/week, with significant differences between groups (P<0.001).

Both groups were able to maintain their body weight during WL maintenance, without significant differences between groups. However, the rapid WL group experienced a significant increase in FFM (kg) with WL maintenance (P<0.001), with significant differences between groups (P=0.010). However, no significant differences between groups were found between baseline and WL maintenance for body weight or composition (see Table 2).

RMR

Groups differed significantly in RMR at the end of WL (P=0.008) and at end of WL maintenance (P=0.001), even after adjusting for FFM (kg) (P=0.010 and P=0.005, respectively). RMR decreased significantly with WL in the rapid group only (P<0.001), and increased significantly with WL maintenance in this group only (P=0.010). No significant differences, within or between groups, were seen when comparing RMR at the end of WL maintenance with baseline (see Table 2).

Exercise efficiency

The rapid WL group experienced a significant increase in NE at 10 watts (P=0.001), and a trend towards an increase at 25 watts (P=0.018) with WL. Differences between groups at the end of WL were significant for 10 and 25 watts (P=0.002 and P=0.003, respectively). With WL maintenance, NE at all levels increased significantly in the gradual WL group (P=0.006, P=0.001, P<0.001 for 10, 25, and 50 watts, respectively), and differences between groups were significant (P=0.002, P=0.004, P=0.008 for 10, 25, and 50 watts, respectively). However, no significant differences between groups were found when comparing changes in NE from baseline to end of WL maintenance (see Table 2).

Appetite feelings

A significant increase in fasting hunger was observed with WL in the gradual group only (P=0.011), with significant differences between groups (P=0.004) (see Figure 2). During WL maintenance, there was a significant increase in fasting hunger in the rapid WL group only (P<0.001). However, differences between groups were not significant. When comparing baseline with WL maintenance, both groups experienced a significant and similar increase in fasting hunger (rapid, P=0.006 and gradual, P<0.001). The rapid WL group experienced a significant decrease in fasting PFC with WL (P=0.003) and differences between groups were significant (P=0.003). During WL maintenance, a significant increase in fasting PFC was observed in the rapid WL group only (P=0.003), with no significant differences between groups. Changes in fasting PFC from baseline to end of WL maintenance were not significantly different, either within or between groups.

Postprandial hunger decreased and postprandial fullness increased significantly with WL in the rapid WL group only (P=0.003 and P<0.001, respectively), and differences between groups were significant (P=0.007 and P=0.016, respectively). After WL maintenance, no significant differences were seen, within or between groups, in postprandial hunger or fullness, either when comparing with end of WL phase or with baseline. Both postprandial desire to eat and PFC decreased significantly with WL in the rapid WL group only (P=0.004 and P=0.001, respectively), and there was a tendency for the postprandial desire to eat (P=0.022) to be lower and postprandial PFC significantly lower (P=0.007) when compared with gradual WL group. After WL maintenance there were no significant differences in postprandial desire to eat or PFC within or between groups, either when comparing with end of WL phase or with baseline.

Appetite-regulating hormones

There were no significant differences in basal AG, CCK, and active GLP-1 over time within or between groups (see Figure 3). Both groups experienced a significant and similar reduction in basal total PYY with WL (P=0.016 and P=0.003 for the rapid and gradual WL groups, respectively). After WL maintenance, basal total PYY increased in the gradual WL group (P=0.007) only, but with no significant differences between groups. Basal insulin was significantly and similarly reduced with WL in both groups (P<0.001 for both). With WL maintenance, basal insulin increased significantly only in the rapid WL group (P=0.016), but with no significant differences between groups. No significant differences, within or between groups, were seen when comparing basal total PYY or insulin at the end of WL maintenance with baseline.

There was a tendency towards a decrease in postprandial AG with WL in the rapid group only (P=0.017), but with no significant differences between groups. However, after WL maintenance, the rapid WL group experienced a significant increase in postprandial AG (P<0.001), and differences between groups were significant (P=0.001). Postprandial CCK decreased significantly with WL in the rapid WL group only (P=0.001), but with no significant differences between groups. With WL maintenance, postprandial CCK increased significantly in the rapid WL group only, (P=0.001), but with no significant differences between groups. No significant differences, within or between groups, were seen when comparing postprandial AG and CCK at the end of WL maintenance with baseline.

There were no significant differences in postprandial total PYY or active GLP-1 over time within or between groups.

Both groups experienced a significant and similar decrease in postprandial insulin with WL (P<0.001 for both). With WL maintenance, no significant difference in postprandial insulin was seen within or between groups. However, postprandial insulin was significantly and similarly lower in both groups at the end of WL maintenance when compared with baseline (P<0.001 for both groups).

Discussion

In this study, changes in body composition were not significantly different after a similar WL (≈9%) achieved either rapidly or gradually. Losing weight gradually, however, prevented the reduction in RMR and the increase in ExEff, observed with rapid WL. On the other hand, changes in appetite were more favorable in the rapid WL group during negative energy balance (EB). However, these differences were no longer apparent after weight stability.

In contrast to the majority of the literature (11, 15, 19), a larger loss of FFM with rapid WL was not observed in this study. In their systematic review, Chaston et al. (2007) concluded that WL rate had a significant impact on the amount of weight lost as FFM (11). However, this needs to be interpreted with caution, given the heterogeneity of studies included and the fact that the amount of WL was not controlled. Coxon et al. (1989) designed an intervention to achieve a WL of 1.9 *vs* 1.1 kg/week, over 8 weeks (24). As expected, the rapid WL group lost a larger amount of weight, and, thus, had also a larger reduction in FFM. However, after adjusting for WL, differences between groups disappeared. More recent studies designed to look at the impact of WL rate on long-term WL maintenance have produced contradictory findings (18, 19). Purcell et al. (2014) reported no differences in FFM loss after rapid (1.2 kg/week) or gradual WL (0.4 kg/week) (18), while Vink et al. (2016) showed a significantly higher FFM% loss with rapid WL (1.8 *vs* 0.6 kg/week) (19). Differences in WL rate may account for some of the discrepancies.

Ketogenic diets are known to lead to a large loss of total body water (TBW) due to glycogen depletion (25), which may bias body composition results. It is possible that part of the reduction in FFM seen in our rapid WL group reflects loss of TBW and, as such, that losing weight fast may be associated with a lower loss of lean tissue. The significant increase in FFM seen during the refeeding period, in the rapid WL group only may, again, partially reflect the increase in TBW that follows glycogen repletion.

The dietary protocol chosen to induce a rapid WL did not cover the daily protein recommendations of the European Food Safety Authority to prevent muscle mass loss (75 g/day) (26). However, the daily protein intake in this group (57 g/day) was, apparently, enough to prevent significant differences in muscle mass loss when compared with the other group, which had a protein intake of more than double (122 g/day).

Losing weight rapidly was associated with a significant reduction in RMR, even after adjusting for FFM. Knowing that FFM is the main determinant of RMR (27), and that in the present study no significant differences between groups were seen in FFM loss, the difference in RMR between groups was unexpected. Thus, the decrease in RMR noticed only in the rapid WL group suggests a metabolic adaptation to rapid WL (28). Similarly, Valtueña and colleagues (1995) also reported that the drop in RMR observed after 1 month VLCD was larger than the loss of FFM (12). Contrary to our findings, Coxon and colleagues (1989) found a significant reduction in RMR after rapid WL, but not after adjusting for FFM loss (24). The duration of dietary restriction may play a role, given that our rapid WL group was on diet for 4 weeks, while in Coxon et al. (1989), subjects were on diet for 8 weeks. Moreover, RMR recovered in our rapid WL group after a period of refeeding, and this recovery was significantly different between groups, even after adjusting for FFM. Foster et al. (1990) reported similar findings, with RMR normalized to FFM partially recovering after a refeeding period (29).

An increase in ExEff with diet-induced WL has been previously reported (30). A significant increase in ExEff at 10 and a trend towards an increase at 25 watts were seen with WL in the rapid group only, while with WL maintenance, an increase in ExEff at all levels was seen in the gradual group only. Even though the reasons for these differences are unknown, macronutrient composition may play a role (31). The isocaloric substitution of CHO by protein was previously shown to increase exercise-induced energy expenditure (31). In the present study, the gradual group had a higher protein intake (g/day) compared with the other group, which might have prevented the increase in ExEff after WL seen in participants who lost weight rapidly. After WL maintenance, the gradual WL group had a marked reduction in protein intake, which might have contributed to the increase in ExEff seen during that phase.

Diet-induced WL has been shown to be associated with a compensatory increase in hunger (32), and concomitant changes in appetite-mediating hormones (33, 34), which encourage weight regain (4, 5). Changes in subjective appetite were more favorable in our rapid WL group. This is in line with the findings from Purcell et al. (2014), where feelings of PFC increased with WL (15%) in the gradual (WL over 36 weeks), but not rapid (WL over 12 weeks) group (18). The more favorable changes in appetite in our rapid WL group are likely to be due to the appetite suppressant effect of ketosis (35, 36). This is further strengthened by the fact that after WL maintenance (and no ketosis) fasting hunger feelings were significantly higher compared with baseline in both groups. Moreover, all the differences between groups in appetite, measured at the end of WL, disappeared after refeeding and weight stabilization. Adding to this, the study of Sumithran et al. (2013), where people with obesity underwent an 8-week ketogenic VLCD (10% WL), followed by 2 weeks of gradual refeeding, reported, similarly to us, a significant increase in fasting hunger with refeeding (36).

The significant increase in fasting hunger noticed in our gradual WL group follows what is expected with non-ketogenic diet-induced WL (4). Doucet and colleagues (2000) reported an increase in fasting hunger, desire to eat and PFC with a 15-week energy restriction (-700 kcal/day) inducing a 10% WL (37). The fact that we have not found in our gradual WL group differences in other appetite feelings besides hunger might be related to differences in the duration of the WL intervention between studies (8 *vs* 15 weeks, respectively).

In this study, WL rate did not affect the concentration of appetite-regulating hormones. Our results reflect those by Sumithran et al. (2013), who reported no significant changes in basal or postprandial AG with WL, but a significant increase with refeeding (36). Further, they also observed that basal and postprandial PYY and insulin, and basal CCK and GLP-1 concentrations decreased significantly with WL, and did not change with WL maintenance (36). The slightly different outcomes may be due to the fact that our group had 4 weeks of WL maintenance while Sumithran's had 2 only, which might not have been long enough to induce hormonal changes. The fact that differences between groups in subjective feelings of appetite were seen in the absence of differences in appetite-related hormones is not completely unexpected, given that previous research has shown that these two variables are not always correlated (38).

The absence of differences in the activation or strength of compensatory mechanisms between groups, once EB was reestablished, is in line with previous RCTs that show that WL rate has no impact on long-term WL maintenance (18, 19).

The main strength of this study is its design, randomized clinical trial. Second, compliance was monitored over time and was excellent. Third, with an intervention period of either 4 or 8 weeks, all women were tested in the same phase of the menstruation cycle, preventing a potential impact of menstrual cycle phase on RMR and appetite (39, 40). Forth, measurements were taken both, immediately after WL, and after WL maintenance, enabling us to look at the impact of WL rate independently of negative EB and ketosis. Fifth, reference methods were used to measure all variables. Finally, both groups lost a similar amount of weight. However, both groups lost weight relatively fast, so the gradual WL group was not within the present recommendations (0.5-1 kg/week). Moreover, a relatively low number of participants is a potential relevant limitation, given that trends for differences in age and gender among the two groups did not reach statistical significance likely because of limited sample size. Finally, the short follow-up period might have not been enough to identify possible differences between groups during the WL maintenance phase.

In conclusion, WL rate does not seem to have a significant impact on body composition, metabolism or appetite, once participants are in EB and not ketotic. However, gradual WL prevented the acute reduction in RMR and increase in ExEff observed with rapid WL, even though acute changes in appetite were more favorable in the rapid WL group, probably related to the fact that this group followed a ketogenic diet. In clinical practice, and given that rate of WL has also been shown not to have a significant impact on long-term WL maintenance (18, 19), participants could potentially be advised to follow a diet with the rate that is for them easier to comply with. Still, more RCT's, with larger sample sizes and different rates of WL, including the present recommendations are needed.

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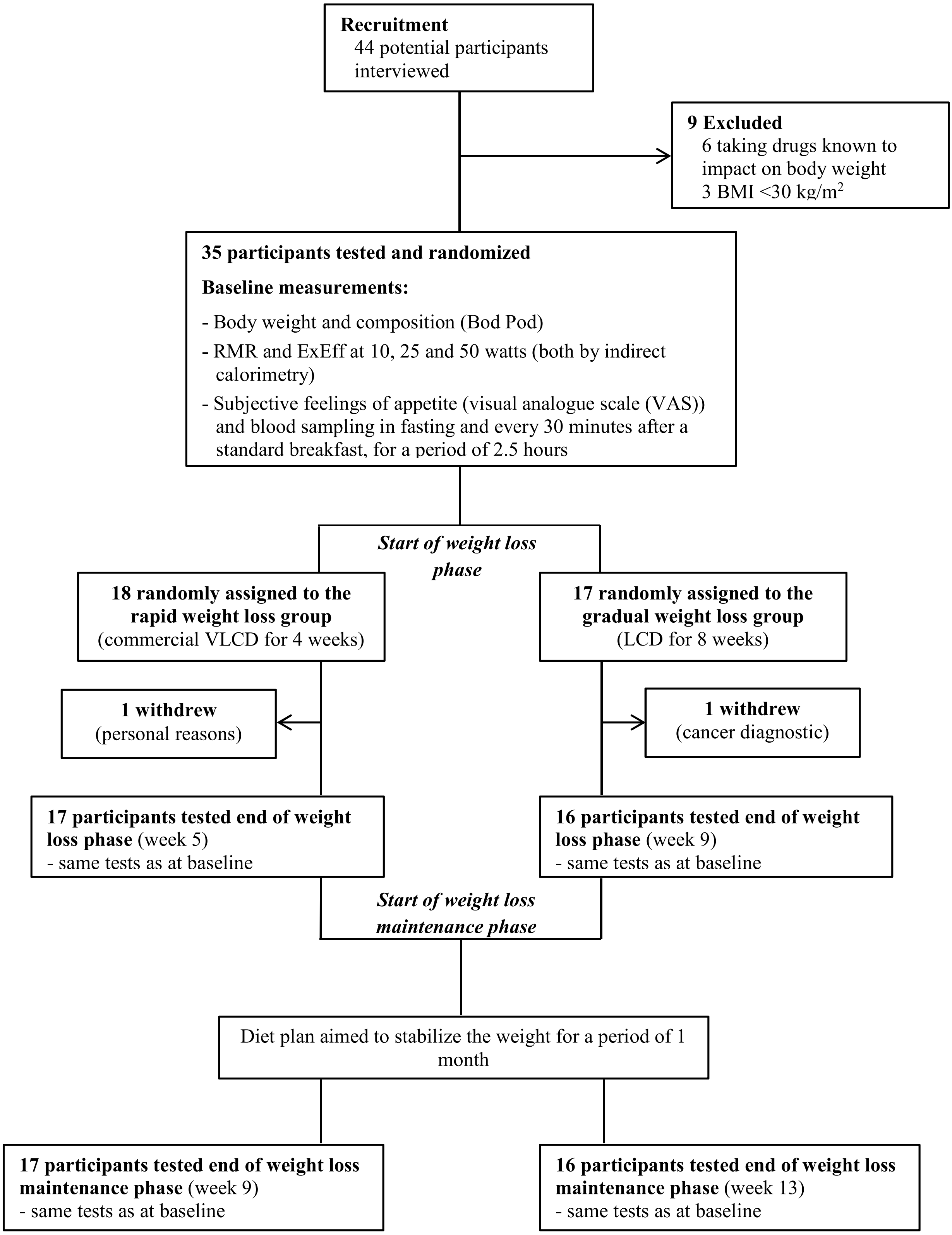
References

1. WHO. Global Health Observatory (GHO) data 2015 [Available from: http://www.who.int/gho/ncd/risk_factors/obesity_text/en/.
2. Pi-Sunyer FX. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert panel on the identification, evaluation, and treatment of overweight in adults. Am J Clin Nutr. 1998;68(4):899-917.
3. Ayyad C, Andersen T. Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999. Obes Rev. 2000;1(2):113-9.
4. Sumithran P, Proietto J. The defence of body weight: a physiological basis for weight regain after weight loss. Clin Sci (Lond). 2013;124(4):231-41.
5. Greenway FL. Physiological adaptations to weight loss and factors favouring weight regain. Int J Obes (Lond). 2015;39(8):1188-96.
6. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med. 1995;332(10):621-8.
7. Rosenbaum M, Vandenborne K, Goldsmith R, Simoneau JA, Heymsfield S, Joanisse DR, et al. Effects of experimental weight perturbation on skeletal muscle work efficiency in human subjects. Am J Physiol Regul Integr Comp Physiol. 2003;285(1):R183-92.
8. Doucet E, Cameron J. Appetite control after weight loss: what is the role of bloodborne peptides? Appl Physiol Nutr Metab. 2007;32(3):523-32.
9. Vincent RP, Ashrafian H, W. lRC. Mechanisms of disease: the role of gastrointestinal hormones in appetite and obesity. Nat Clin Pract Gastroenterol Hepatol. 2008;5(5):268-77.
10. Lean ME, Malkova D. Altered gut and adipose tissue hormones in overweight and obese individuals: cause or consequence? Int J Obes. 2015:1-11.
11. Chaston TB, Dixon JB, O'Brien PE. Changes in fat-free mass during significant weight loss: a systematic review. International Journal of Obesity. 2007;31(5):743-50.
12. Valtueña S, Blanch S, Barenys M, Solà R, Salas-Salvadó J. Changes in body composition and resting energy expenditure after rapid weight loss: is there an energy-metabolism adaptation in obese patients? Int J Obes Relat Metab Disord. 1995;19(2):119-25.
13. Wadden TA, Foster GD, Letizia KA. One-year behavioral treatment of obesity: comparison of moderate and severe caloric restriction and the effects of weight maintenance therapy. J Consult Clin Psychol. 1994;62(1):165-71.
14. Sénéchal M, Arguin H, Bouchard DR, Carpentier AC, Ardilouze JL, Dionne IJ, et al. Effects of rapid or slow weight loss on body composition and metabolic risk factors in obese postmenopausal women. A pilot study. Appetite. 2012;58(3):831-4.
15. Arguin H, Bouchard DR, Labonté M, Carpentier A, Ardilouze JL, Dionne IJ, et al. Correlation between the rate of weight loss and changes in body composition in obese postmenopausal women after 5 weeks: a pilot study. Appl Physiol Nutr Metab. 2008;33(2):347-55.
16. Casazza K, Brown A, Astrup AB, F., Baum C, Brown MB, Dawson J, et al. Weighing the evidence of common beliefs in obesity research. Crit Rev Food Sci Nutr. 2015;55(14):2014-53.
17. Nackers LM, Ross KM, Perri MG. The association between rate of initial weight loss and long-term success in obesity treatment: does slow and steady win the race? Int J Behav Med. 2010;17(3):161-7.
18. Purcell K, Sumithran P, Prendergast LA, Bouniu CJ, Delbridge E, Proietto J. The effect of rate of weight loss on long-term weight management: a randomised controlled trial. Lancet Diabetes Endocrinol. 2014;2:954-62.
19. Vink RG, Roumans NJ, Arkenbosch LA, Mariman EC, van Baak MA. The effect of rate of weight loss on long-term weight regain in adults with overweight and obesity. Obesity (Silver Spring). 2016;24(2):321-7.

20. Scheers T, Philipaerts R, Lefevre J. Patterns of physical activity and sedentary behavior in normal-weight, overweight and obese adults, as measured with a portable armband device and an electronic diary. Clinical Nutrition. 2012;31(5):756-64.
21. Compher C, Frankenfield D, Keim N, Roth-Yousey L. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. Journal of the American Dietetic Association. 2006;106(6):881-903.
22. Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. British Journal of Nutrition. 2000;84:405-15.
23. Rehfeld JF. Accurate measurement of cholecystokinin in plasma. Clin Chem. 1998;44(5):991-1001.
24. Coxon A, Kreitzman S, Brodie D, Howard A. Rapid weight loss and lean tissue: evidence for comparable body composition and metabolic rate in differing rates of weight loss. Int J Obes. 1989;13 Suppl 2(Suppl 2):179-81.
25. Yang MU, Van Itallie TB. Composition of weight lost during short-term weight reduction. Metabolic responses of obese subjects to starvation and low-calorie ketogenic and nonketogenic diets. J Clin Invest. 1976;58(3):722-30.
26. EFSA NDA Panel (EFSA Panel on Dietetic Products NaA. Scientific Opinion on the essential composition of total diet replacements for weight control. EFSA Journal. 2015;13(1):3957.
27. Sparti A, DeLany JP, de la Bretonne JA, Sander GE, Bray GA. Relationship between resting metabolic rate and the composition of the fat-free mass. Metabolism. 1997;46(10):1225-30.
28. Luke A, Schoeller DA. Basal metabolic rate, fat-free mass, and body cell mass during energy restriction. Metabolism. 1992;41(4):450-6.
29. Foster GD, Wadden TA, Feurer ID, Jennings AS, Stunkard AJ, Crosby LO, et al. Controlled trial of the metabolic effects of a very-low-calorie diet: short- and long-term effects. Am J Clin Nutr. 1990;51(2):167-72.
30. Doucet E, Imbeault P, St-Pierre S, Alméras N, Mauriège P, Després JP, et al. Greater than predicted decrease in energy expenditure during exercise after body weight loss in obese men. Clin Sci (Lond). 2003;105(1):89-95.
31. Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. American Journal of Clinical Nutrition. 2000;72(5):1135-41.
32. Ochner CN, Barrios DM, Lee CD, Pi-Sunyer FX. Biological mechanisms that promote weight regain following weight loss in obese humans. Physiol Behav. 2013;120:106-13.
33. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med. 2002;346(21):1623-30.
34. Pfluger PT, Kampe J, Castaneda TR, Vahl T, D'Alessio DA, Kruthaupt T, et al. Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. J Clin Endocrinol Metab. 2007;92(2):583-8.
35. Gibson AA, Seimon RV, Lee CM, Ayre J, Franklin J, Markovic TP, et al. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. Obes Rev. 2015;16(1):64-76.
36. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Ketosis and appetite-mediating nutrients and hormones after weight loss. Eur J Clin Nutr. 2013;67:759-64.
37. Doucet E, Imbeault P, St-Pierre S, Alméras N, Mauriège P, Richard D, et al. Appetite after weight loss by energy restriction and a low-fat diet-exercise follow-up. Int J Obes Relat Metab Disord. 2000;24(7):906-14.
38. Doucet E, Laviolette M, Imbeault P, Strychar I, Rabasa-Lhoret R, Prud'homme D. Total peptide YY is a correlate of postprandial energy expenditure but not of appetite or energy intake in healthy women. Metabolism. 2008;57(10):1458-64.

39. Henry CJ, Lightowler HJ, Marchini J. Intra-individual variation in resting metabolic rate during the menstrual cycle. British Journal of Nutrition. 2003;89(6):811-7.
40. Brennan IM, Feltrin KL, Nair NS, Hausken T, Little TJ, Gentilcore D, et al. Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. Am J Physiol Gastrointest Liver Physiol. 2009;297:G602–G10.

Figure 1. Study diagram



BMI: body-mass index; RMR: resting metabolic rate; ExEff: exercise efficiency; VLCD: very low calorie diet; LCD: Low calorie diet.

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

	Rapid WL group (N=17)	**Gradual WL group** (N=16)	**P-*value***
Age (years)	42.2±10.0	36.2±8.7	0.115
Gender ratio (women : men)	14 : 3	10 : 6	0.219
Body weight (kg)	96.6±12.2	99.4±12.1	0.523
BMI (kg/m^2)	33.4±3.0	33.5±2.6	0.811
Fat Mass (kg)	42.8±8.1	43.2±6.2	0.906
Fat Mass (%)	44.2±4.7	43.6±4.7	0.675
Fat Free Mass (kg)	53.7±7.1	56.3±9.0	0.386
Fat Free Mass (%)	55.8±4.7	56.5±4.7	0.675
RMR (kcal/day)	1319±179	1359±201	0.687

Data presented as mean ± SD. WL: weight loss; BMI: body-mass index; RMR: resting metabolic rate. P-values for comparison between groups at baseline.

Table 2. Changes in anthropometric measurements, RMR, and exercise efficiency in the rapid and gradual weight loss groups

	Rapid WL group						**Gradual WL group**					
	Baseline to end of WL phase	**P-*value***	**End of WL phase to end of WL maintenance phase**	**P-*value***	**Baseline to end of WL maintenance phase**	**P-*value***	**Baseline to end of WL phase**	**P-*value***	**End of WL phase to end of WL maintenance phase**	**P-*value***	**Baseline to end of WL maintenance phase**	**P-*value***
Weight (kg)	-8.9±0.5	<0.001	-0.6±0.5	0.261	-9.4±0.5	<0.001	-9.3±0.5	<0.001	-1.0±0.5	0.044	-10.3±0.5	<0.001
FM (kg)	-6.6±0.4	<0.001	-1.9±0.4	<0.001	-8.5±0.4	<0.001	-7.6±0.4	<0.001	-1.3±0.4	0.003	-8.9±0.4	<0.001
FM (%)	-3.2±0.4	<0.001	-2.0±0.4	<0.001	-5.2±0.4	<0.001	-4.0±0.4	<0.001	-1.1±0.4	0.011	-5.1±0.4	<0.001
FFM (kg)	-2.2±0.3	<0.001	$1.3±0.3^{a}$	<0.001	-0.9±0.3	0.005	-1.7±0.3	<0.001	$0.3±0.3^{a}$	0.427	-1.5±0.3	<0.001
FFM (%)	3.2±0.4	<0.001	2.0±0.4	<0.001	5.2±0.4	<0.001	4.0±0.4	<0.001	1.0±0.4	0.013	5.1±0.4	<0.001
RMR (kcal/day)	$-129.4±31.6^{b; 1}$	<0.001	$87.5±33.1^{c; 2}$	0.010	-41.9±33.1	0.210	$-23.9±32.6^{b; 1}$	0.465	$-57.4±33.3^{c; 2}$	0.091	-81.3±33.3	0.018
NE 10 watts	$0.009±0.002^{d}$	0.001	$-0.003±0.002^{e}$	0.224	0.006±0.002	0.025	$-0.001±0.002^{d}$	0.732	$0.007±0.002^{e}$	0.006	0.006±0.002	0.014
NE 25 watts	$0.009±0.004^{f}$	0.018	$-0.001±0.004^{g}$	0.838	0.008±0.004	0.038	$-0.005±0.004^{f}$	0.143	$0.013±0.004^{g}$	0.001	0.008±0.004	0.045
NE 50 watts	0.002±0.004	0.580	$0.004±0.004^{h}$	0.342	0.006±0.004	0.141	-0.004±0.004	0.307	$0.017±0.004^{h}$	<0.001	0.013±0.004	0.002

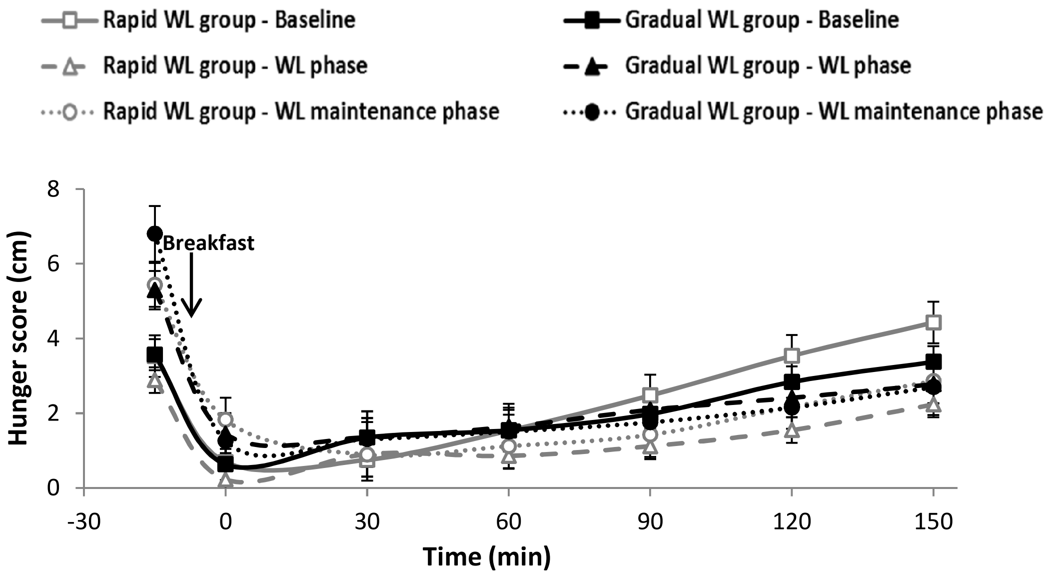
Data presented as mean ± SEM. WL: weight loss; FM: fat mass; FFM: fat free mass; RMR: resting metabolic rate; NE: net efficiency. P-values are for changes in anthropometric measurements, in RMR, and in exercise efficiency between time points within groups. Data were analyzed using linear mixed-effect models, and Bonferroni correction was used for post hoc pairwise comparisons. Significance level was assumed at P-*values*<0.017.

Means sharing the same superscript letter denote significant differences between groups: $^{a, b, g, h}$ P<0.05; $^{c, d, e, f}$ P<0.01.

[1] After adjusting for FFM (in kg) at the end of WL phase (RMR_{FFM} (kcal/day / kg FFM)), there continued to be a significant difference in RMR between groups (P=0.010).

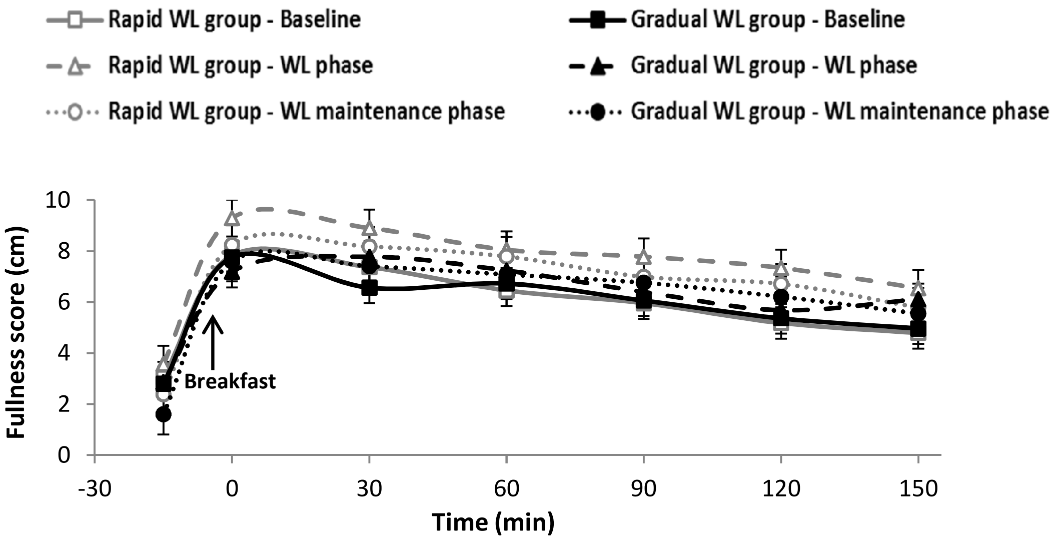
[2] After adjusting for FFM (in kg) at the end of WL maintenance phase (RMR_{FFM} (kcal/day / kg FFM)), there continued to be a significant difference in RMR between groups (P=0.005).

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



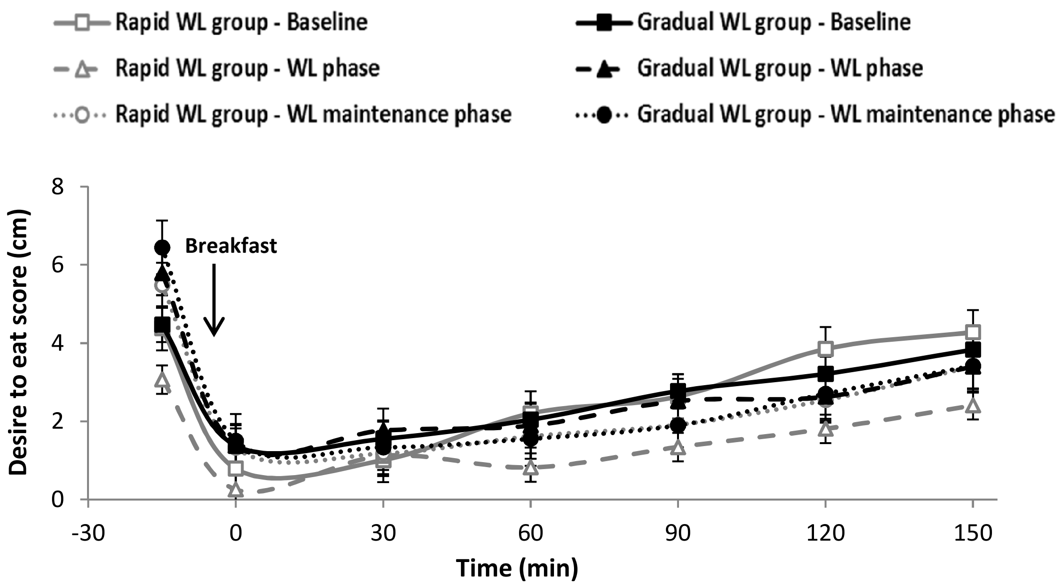
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. Higher numbers indicate greater hunger. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



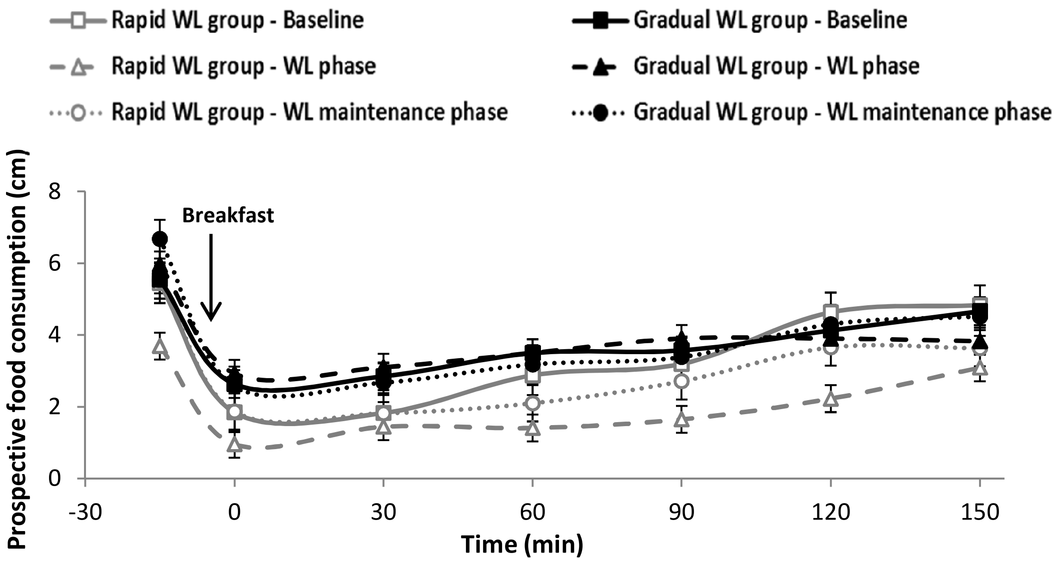
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. Higher numbers indicate greater fullness. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



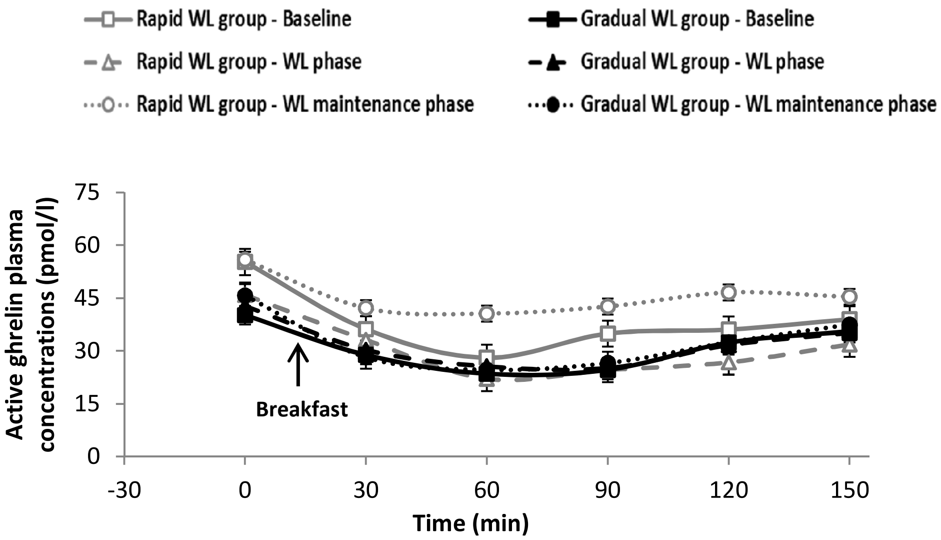
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. Higher numbers indicate greater desire to eat. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



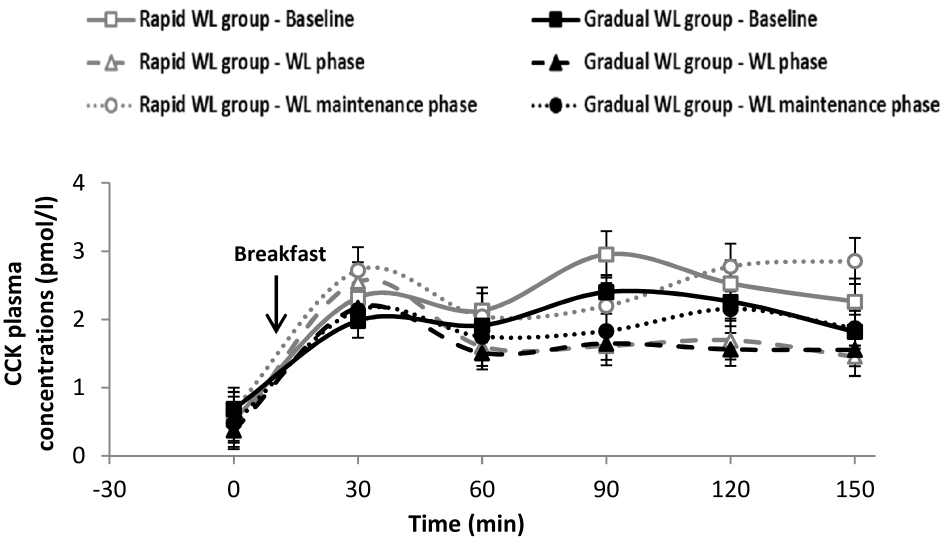
Ratings were based on a visual-analogue scale ranging from 0 to 10 cm. Higher numbers indicate greater prospective food consumption. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



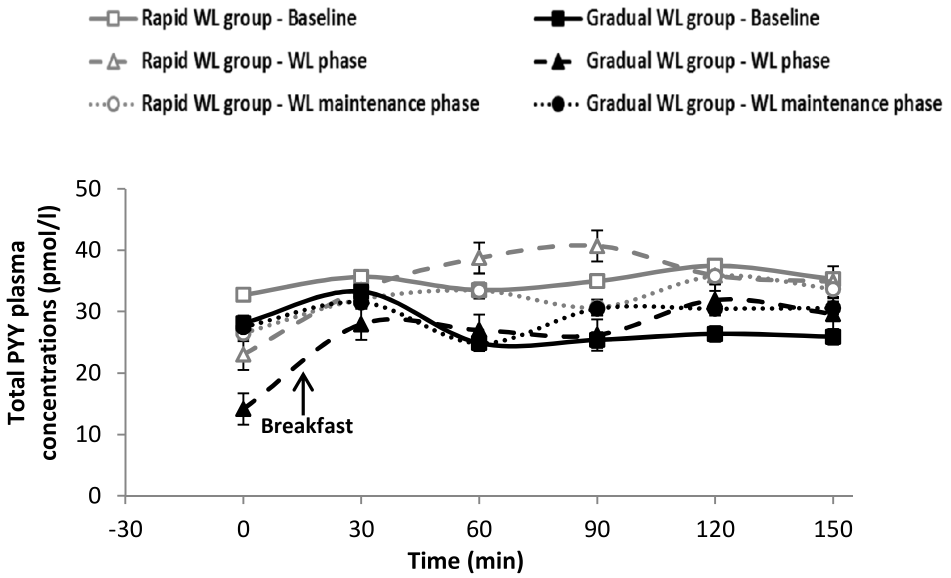
Plasma concentrations (pmol/l) of active ghrelin over time (min) in both groups. In fasting and every 30 minutes after a standard breakfast, for a period of 2.5 hours. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



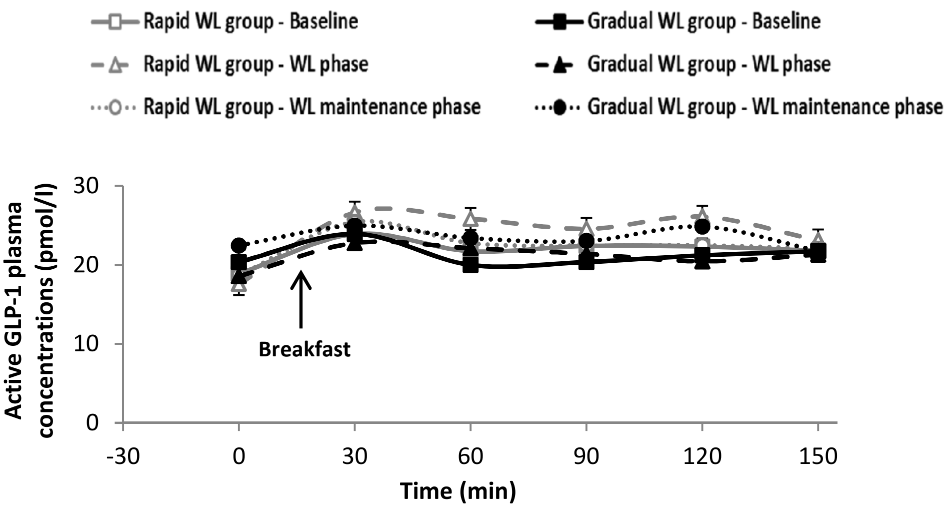
Plasma concentrations (pmol/l) of CCK over time (min) in both groups. In fasting and every 30 minutes after a standard breakfast, for a period of 2.5 hours. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



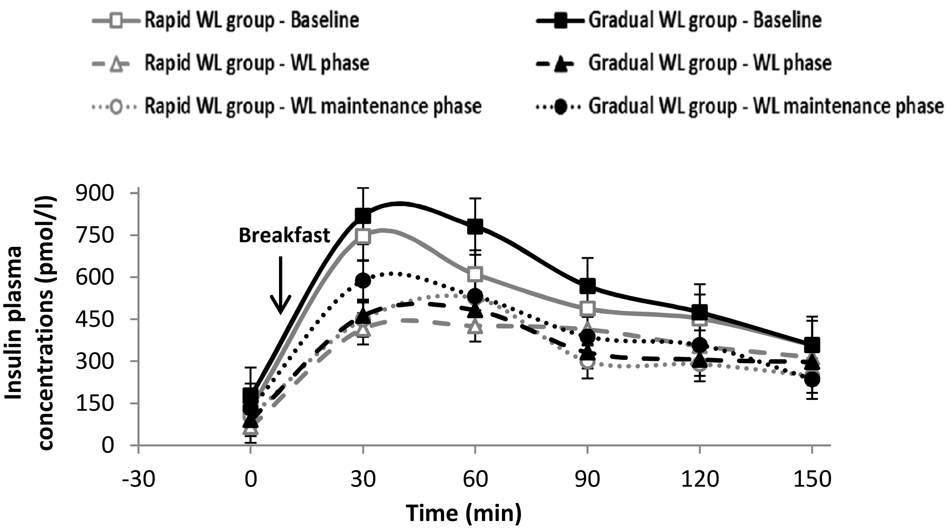
Plasma concentrations (pmol/l) of total PYY over time (min) in both groups. In fasting and every 30 minutes after a standard breakfast, for a period of 2.5 hours. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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. In fasting and every 30 minutes after a standard breakfast, for a period of 2.5 hours. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

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



Plasma concentrations (pmol/l) of insulin overtime (min) in both groups. In fasting and every 30 minutes after a standard breakfast, for a period of 2.5 hours. WL: weight loss. Data presented as mean (± SEM) at baseline, end of weight loss phase, and end of weight loss maintenance phase for both groups.

Table S1. Dietary plan for the rapid weight loss group

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

Table S2. Dietary plan for the gradual weight loss group

Meal	**Women (**1200 kcal/day**)**	**Men (**1500 kcal/day**)**
Breakfast	1 Milkshake (Product)	1 Milkshake (Product)
Lunch	1 Soup (Product) + 1 toast + 15g low fat cheese (9%) **or** 15 g ham	1 Soup (Product) + 1 Cereal bar (Product)
Snack	1 Cereal bar (Product)	1 Cereal bar (Product)
Dinner	200 g of low fat fish **or** meat **or** 3 eggs + 50g of cooked pasta/rice **or** 80 g of raw potatoes **or** 30 g of bread + 165 g of low-starch vegetables (described above) + 1 (medium size) fruit (pear, apple, orange, peach)	250 g of low fat fish **or** meat or 3 eggs + 50 g of cooked pasta/rice **or** 80 g of raw potatoes **or** 30 g of bread + 165 g of low-starch vegetables (described above) + 1 (medium size) fruit (pear, apple, orange, peach)
Snack	200 ml of low fat milk (0.1%) **or** 125 g of low fat yogurt + 1 toast + 15 g low fat cheese (9%) **or** 15 g ham	200 ml of low fat milk (0.1%) **or** 125 g of low fat yogurt + 2 toasts + 45 g low fat cheese (9%) **or** 45 g ham

Table S3. Energy intake and macronutrient composition of the diet in the rapid and gradual weight loss groups

	Rapid WL group			Gradual WL group			P-*value**
	2nd week	4th week (end of WL phase)	P-*value*	4th week	8th week (end of WL phase)	P-*value*	
Energy (kcal/day)	590.0±23.8	593.1±23.8	0.762	1332.1±24.6	1318.0±24.6	0.191	<0.001
Protein (g)	57.6±2.7	57.1±2.7	0.698	122.3±2.7	121.6±2.7	0.591	<0.001
Protein (%)	39.1±0.3	38.5±0.3	0.099	36.9±0.3	37.0±0.3	0.689	0.001
Fat (g)	9.9±0.9	10.0±0.9	0.942	27.1±0.9	26.2±0.9	0.360	<0.001
Fat (%)	15.1±0.4	15.1±0.4	1.000	18.1±0.4	17.5±0.4	0.265	<0.001
CHO (g)	59.0±2.1	57.8±2.1	0.443	136.5±2.2	135.5±2.2	0.545	<0.001
CHO (%)	40.0±0.3	39.0±0.3	0.016	41.1±0.4	41.4±0.4	0.525	<0.001
Fiber(g)	17.2±0.7	17.9±0.7	0.082	24.5±0.7	23.0±0.7	0.001	<0.001
Fiber (%)	5.8±0.1	6.0±0.1	0.002	3.7±0.1	3.5±0.1	0.009	<0.001

Data presented as mean ± SEM. P-values are for changes in energy intake and macronutrient composition of the diet between time points within groups. WL: weight loss; CHO: carbohydrates. *P-values for comparison between groups at the end of WL phase. 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.

Table S4. Changes in physical activity levels in the rapid and gradual weight loss groups

	Rapid WL group						Gradual WL group					
	Baseline to end of WL phase	**P-*value***	**End of WL phase to end of WL maintenance phase**	**P-*value***	**Baseline to end of WL maintenance phase**	**P-*value***	**Baseline to end of WL phase**	**P-*value***	**End of WL phase to end of WL maintenance phase**	**P-*value***	**Baseline to end of WL maintenance phase**	**P-*value***
Sedentary (min)	33.3±34.0	0.401	-113.3±46.7	0.018	-80.0±44.3	0.076	-26.3±31.0	0.505	-1.5±32.4	0.491	-27.8±31.0	0.333
Light (min)	10.5±12.0	0.386	6.3±17.0	0.345	16.8±16.2	0.306	1.6±10.6	0.433	12.2±11.2	0.282	13.8±10.6	0.202
Moderate (min)	-8.8±7.1	0.221	23.3±9.7	0.019	14.5±9.2	0.119	-6.8±6.5	0.297	7.8±6.8	0.257	0.9±6.5	0.382
Vigorous & Very vigorous (min)	-1.3±0.7	0.049	-0.5±0.9	0.367	-0.8±0.8	0.397	0.8±0.6	0.216	0.2±0.6	0.350	1.0±0.6	0.128
Steps/day	-517.9±639.3	0.458	392.4±892.6	0.333	-125.4±849.1	0.546	-512.5±576.4	0.523	773.1±604.4	0.208	260.6±576.4	0.417

Data presented as mean ± SEM. P-values are for changes in physical activity levels over time within each group or differences between groups. WL: weight loss. 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. Bonferroni correction was used for post hoc pairwise comparisons. Significance level was assumed at P-*values*<0.017.