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Siervo, M, Faber, P, Lara, J et al. (7 more authors) (2015) Imposed rate and extent of weight loss in obese men and adaptive changes in resting and total energy expenditure. Metabolism, 64 (8). pp. 896-904. ISSN 0026-0495

https://doi.org/10.1016/j.metabol.2015.03.011

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Manuscript Number: METABOLISM-D-14-00727R3

Title: Imposed rate and extent of weight loss in obese men and adaptive changes in resting and total energy expenditure

Article Type: Research Paper

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*Highlights (for review)*

- Different rates of weight loss induced a similar decline in total EE
- Adaptive thermogenesis explained ~6% of the decline in total EE after weight loss
- Adaptive changes in resting EE were associated with rate of weight loss
IMPOSED RATE AND EXTENT OF WEIGHT LOSS IN OBESE MEN
AND ADAPTIVE CHANGES IN RESTING AND TOTAL ENERGY
EXPENDITURE

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Short title: Rate and extent of weight loss and changes in energy expenditure

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The material presented in this manuscript is original and it has not been submitted for
publication elsewhere while under consideration for Metabolism – Clinical and Experimental

The authors have no conflicts of interest to declare

Keywords: Obesity, weight loss, energy expenditure, metabolic adaptation

Abbreviations: weight loss= WL; energy expenditure= EE; very low calorie diet= VLCD;
low calorie diet= LCD; fat mass= fat mass; fat free mass= FFM; dietary induced
thermogenesis= DIT; Rowett Institute of Nutrition and Health= RINH; doubly labelled
water= DLW; caloric restriction= CR.

Abstract: 243; Main text: 4190; References: 50; Tables: 4; Figures: 3; OSM: 1
Abstract

Objectives: Weight loss (WL) is associated with a decrease in total and resting energy expenditure (EE). We aimed to investigate whether 1) diets with different rate and extent of WL determined different changes in total and resting EE and if 2) they influenced the level of adaptive thermogenesis, defined as the decline in total or resting EE not accounted by changes in body composition.

Methods: Three groups of six, obese men participated in a total fast for 6 days to achieve a 5%WL and a very low calorie (VLCD, 2.5MJ/day) for 3 weeks or a low calorie (LCD, 5.2MJ/day) diet for 6 weeks to achieve a 10%WL. A four-component model was used to measure body composition. Indirect calorimetry was used to measure resting EE. Total EE was measured by doubly labelled water (VLCD, LCD) and 24-hr whole-body calorimetry (fasting).

Results: VLCD and LCD showed a similar degree of metabolic adaptation for total EE (VLCD=−6.2%; LCD=−6.8%). Metabolic adaptation for resting EE was greater in the LCD (-0.4MJ/day, -5.3%) compared to the VLCD (-0.1MJ/day, -1.4%) group. Resting EE did not decrease after short-term fasting and no evidence of adaptive thermogenesis (+0.4MJ/day) was found after 5%WL. The rate of WL was inversely associated with changes in resting EE (n=30, r=−0.42, p=0.01).

Conclusions: The rate of WL did not appear to influence the decline in total EE in obese men after 10%WL. Approximately 6% of this decline in total EE was explained by mechanisms of adaptive thermogenesis.
1. Introduction

Weight loss (WL) is associated with modifications of fuel oxidation and resting and total energy expenditure (EE). Changes in body composition [i.e., fat mass (FM) and fat free mass (FFM)] explain a large proportion of the decrease in EE, which may be linked to the loss of metabolically active cellular mass, lower dietary induced thermogenesis (DIT) and energy cost of physical activity. The residual EE not accounted for by the observed body composition and metabolic changes could derive from modifications of the efficiency and activity of metabolic, endocrine and autonomic pathways (i.e., adaptive thermogenesis).

However, the occurrence of adaptive metabolic changes during WL is not a consistent finding across WL studies. These differences could be explained by the different approaches used to quantify metabolic adaptation, such as application of different for the measurement of body composition and/or energy expenditure, as well as to the characteristics of the study population (adiposity, age, health status), degree of negative energy balance and duration of the WL interventions. Specifically, the level of the negative energy balance (i.e., very low calorie diet (VLCD), low calorie diet (LCD)), the macronutrient composition of the hypocaloric diets (i.e., high protein, low fat) and the type (i.e., resistance, aerobic) and intensity (i.e., workload and frequency) of physical activity can influence the rate of weight change (how quickly you lose weight over time, kg/d), and amount of WL (total loss in kg, or relative loss %).

Several studies have investigated the effects of fasting or energy-restricted diets on body composition and EE in obese subjects. These studies aimed primarily at testing the effects of the extent of WL on EE; however, none of them has so far compared the effects of diets inducing different rates of WL on resting and total EE in controlled, experimental settings. The majority of these studies have been conducted in free-living conditions, which
may have contributed to the inconsistent results and, consequently, fuelled the debate on the existence and physiological relevance of adaptive thermogenesis associated with WL\textsuperscript{[19, 20]}.

We hypothesised that the rate of WL may represent the primary determinant of the decline in resting and total EE in obese subjects losing a similar amount of body weight. We predicted that a greater level of negative energy balance could be associated with a greater loss of FFM, which may result in greater adaptive changes in both resting and total EE.

This analysis aimed to investigate whether three groups of obese men, exposed to different levels of negative energy balance (fasting, very low calorie diet (VLCD, 2.5MJ/day) and low-calorie diet (LCD, 5.2MJ/day)) in experimental controlled conditions, were characterised by distinct changes in resting and total EE after losing a similar amount of body weight (5% and 10%WL). The study also provided the opportunity to test if the rate of WL and weight lost as FFM were associated with the level of adaptive thermogenesis.

2. Materials and methods

2.1 Subject characteristics

Eighteen (n=6 in each group), healthy, non-smoking, obese (body mass index (BMI) = 33-40 kg/m\textsuperscript{2}) male subjects, aged between 19-55 years, were recruited. Subjects were not following any special diet and were not prescribed any regular medication. A description of the inclusion and exclusion criteria is reported in the Online Supplementary Material. The study was approved by the Grampian Research Ethics Committee. Written informed consent was obtained.

2.2 Experimental design

Subjects were non-randomly allocated to three WL interventions (fasting, VLCD, LCD) with a similar study design as previously described\textsuperscript{[21]}. A description of the study protocol for each WL intervention is provided in the online supplementary material (Figure S1-S3).
Briefly, during the 6-day baseline period subjects consumed a fixed maintenance diet (13% protein, 30% fat and 57% carbohydrate). After the 7-day baseline period, each group followed the specific diet to lose 5% and 10% of their baseline body weight. However, the duration of the fasting was of 6 days as ethical constraint allowed to fast subjects to lose 5% of their baseline body weight. The duration of the WL phases to achieve a 10%WL was of 3 and 6 weeks for the VLCD and LCD groups, respectively. Throughout the study, participants were residential in the Human Nutrition Unit at the Rowett Institute of Nutrition and Health (RINH), Aberdeen, UK. All food and drinks consumed by each participant during the study were supplied by the dietetics staff in the Unit. The participants were requested not to undertake any other strenuous physical activity during the study and they were asked to record their individual exercise sessions.

2.3 Energy and dietary intake

Energy intake (EI) was measured daily, based on the recorded weighed intakes of food and drink and using values from McCance and Widdowson, ‘The composition of foods’ [22]. During starvation, the participants had access to water only. The VLCD comprised: daily weight 642g, energy 2.55kJ/g, protein 49.4g (32%), carbohydrate 52.8g (35%), and fat 23.1g (33%). The LCD comprised: daily weight 1260g, energy 5.2kJ/g, protein 50.3g (17%), carbohydrate 155.7g (50%), and fat 45.4g (33%). Further details are provided in Table S1 of the Online Supplementary Material. Diets and recipes are available upon request. The Department of Health and Social Security (1987) guidelines were adopted for the design of the WL diets and ensure a balanced intake of protein, minerals and vitamins [23].

2.4 Resting Energy Expenditure

REE was measured at baseline and at the end of each WL phase (5% and 10%WL) by indirect calorimetry over 30–40 min using a ventilated hood system (Deltatrac II, MBM-200, Datex Instrumentarium Corporation, Finland). During the measurement, subjects lay on a bed
in a thermo-neutral room and were instructed to lie still but not to fall asleep. Resting EE was calculated from minute-by-minute data using the mean of 15 min of stable measurements, with the first and last 5 min excluded. The equations of Elia and Livesey\textsuperscript{24} were used to derive resting EE. Details of calibration burns and repeatability testing have been described previously\textsuperscript{25}.

2.5 Total Energy expenditure

Measurement of total EE by whole-body indirect calorimetry: Subjects in the fasting group resided in the whole-body room calorimeter for three days during the fasting phase (evening of day 8 to morning of day 12). The study was conducted in the 2 whole-body indirect calorimeters at RINH, which are identical in design and layout. A previous report described the chambers, their initial calibration, and ongoing system checks\textsuperscript{26}. The gas analyzers were calibrated before every run with the use of an atmospheric gas, nitrogen, and a span scaling gas. The span gases were checked by comparison with alpha standard gases, corrected to standard temperature and pressure (British Oxygen Company, Guilford, United Kingdom). During the run, the analyzers were corrected for drift every 3 h with the use of atmosphere as a reference. As previously described, oxygen consumption and carbon dioxide production were estimated by using the rapid-response calculations of Brown et al\textsuperscript{27}. EE was calculated from oxygen and carbon dioxide exchanges and urinary nitrogen excretion by using the values of Livesey and Elia\textsuperscript{24} for volumes of oxygen consumed per oxidized gram of protein, fat, and carbohydrate and the associated respiratory quotients.

Measurement of total EE by doubly labelled water technique: Subjects in both VLCD and LCD groups were dosed orally with doubly labelled water on the morning of day 7. Subjects received a bolus dose of DLW to estimate total EE during the following 10-day period. At 07:00 hours, subjects were woken up and asked to empty their bladders and were weighed. At 09:00 hours, they provided a urine sample to be used as baseline, along with two further
background samples to provide information on the pre-dose isotopic enrichment of the subject's body water pools. Immediately after providing the 09:00 hour sample, each subject was asked to consume a pre-prepared dose of $^2\text{H}_2^{18}\text{O}$. The dose, bottle and straw used for dose consumption were weighed before and after dosing to two decimal places to allow for accurate determination of the quantity consumed by the subject. Subjects also consumed 100 ml of tap water after the dose to prevent isotope loss from the subject's buccal cavity. The dose levels were: 0.15g $^2\text{H}_2\text{O}$ /kg body weight and 0.9g $^2\text{H}_2^{18}\text{O}$ /kg body weight. Each dose was prepared, sealed and autoclaved the day before dosing. Subjects then collected aliquots of urine at 4, 5 and 6 hours after dosing to enable plateau to be individually measured using the "slope intercept" method. Subjects continued to collect an aliquot sample at 11.00 hours for the next 10 days. Subjects in the VLCD groups received 2 doses over a period of 20 days (day 7 and day 18). Subjects in the LCD groups received 4 doses over a period of 40 days (day 7, day 18, Day 28, day 39). Samples were immediately frozen at -20°C after collection. Urine samples were collected for a multi-point stable-isotope analysis using gas isotope ratio mass-spectrometry. Urine isotope enrichments were determined using the platinum equilibration technique \[28\] for $^2\text{H}$ and the CO$_2$ equilibration technique \[29\] for $^{18}\text{O}$. The log-transformed data of enrichment by time was extrapolated back to time zero, giving a theoretical enrichment at time zero, which provided information on the individual's size of the body water pool assuming the dilution principle. Isotopic enrichment of the post-dose urine samples was analysed relative to the original background amounts. Isotope turnover rates, water pool sizes and CO$_2$ production were calculated using the multipoint method \[30\]. Total EE was calculated from CO$_2$ production using classical respirometry formulae and measured food quotient from the provided diets.

2.6 Measurement of body composition
Subject height was measured to the nearest 0.1 cm (Holtain Ltd. Crymych, Dyfeld, Wales, UK) and body weight was measured each morning to the nearest 50 g (DIGI DS-410, CMS Weighing Equipment London, UK). The four-compartment model of body composition as described by Fuller et al[31] was used to measure FM and FFM. Total body water (TBW-kg) was measured by deuterium dilution (D$_2$O) as described by Pullicino et al[32]. Bone mineral mass (BMM-kg) was measured by dual energy X-ray absorptiometry scanning (DEXA; Norland XR-26, Norland corporation, Wisconsin, USA). Body volume and density was measured using a system of air displacement (Bod Pod, Life Measurement Instruments, Concord, Connecticut, USA). The measurement protocols of the various body composition methods have been previously reported[21].

2.7 Statistical analysis

Data are reported as mean and standard deviation. Error bars in the figures are standard error of means. Multiple linear regression analysis was performed on the pooled baseline data from the three WL interventions (N = 18) to derive sample-specific prediction equations for the estimation of resting and total EE. Resting and total EE were entered as the dependent variables and fat mass (FM, kg) and fat free mass (FFM, kg) were the independent variables. These equations were then used to predict resting and total EE at the end of the weight loss (5%WL, 10%WL) interventions. Absolute and relative differences between the measured and predicted resting and total EE were calculated for each WL group to evaluate the presence of adaptive thermogenesis. Paired t-test was used to test whether within-subject changes in body composition and EE in each WL intervention. Independent t-test was used to test differences between WL interventions. Subjects in the fasting group resided in the 24-hr whole-body indirect calorimetry for 3 days (Day 3, Day 4, Day 5). A linear regression model was fitted to the 3-day calorimetry data to impute the remaining missing data (Baseline, Day 1, Day 2, Day 6). This approach was based on the assumption of a linear

decline in total EE. The intercept (time 0) was used to back-extrapolate baseline total EE and the slope was used to impute the remaining missing data points. One-way repeated-measure analysis of variance was used to determine whether 6-day fasting induced significant changes in total EE. Correlation analysis was performed to assess the association between the rate of WL (kg) and weight lost as FFM with adaptive changes in resting and total EE. SPSS 17 (SPSS for Windows, SPSS Inc, USA) was used for the statistical analysis. The significance cut-off p-value was set at 0.01 to account for multiple comparisons.

3. Results

3.1 Weight loss and changes in body composition

Baseline characteristics of subjects included in the three WL groups are reported in Table 1. Weight loss in the fasting group was 6.0 kg over 6 days. The VLCD group lost 5.2 and 9.2 kg over 11 and 21 days and the LCD group lost 7.2 and 12.6 kg over 21 and 42 days, respectively. Mean rates of WL during the 5% WL period were different between the fasting (-1.01 kg/d), VLCD (-0.52 kg/d) and LCD (-0.35 kg/d) groups. The LCD groups lost more FM after each WL phase compared to the fasting (p<0.01) and VLCD (p<0.01) groups (Table 2). The fraction of FFM to total WL after 5%WL was 46, 30 and 18% for the fasting, VLCD and LCD groups respectively. At 10% WL, the VLCD losses were 20% FFM and 80% FM compared with 9% FFM and 91% FM in the LCD group (Figure S4 of the Online Supplementary Material).

3.2 Changes in total and resting energy expenditure

Changes in total EE measured by DLW at the end of the 10%WL phase were not different between the VLCD (-1.3MJ/day) and LCD (-1.5MJ/day) groups (p>0.05). This corresponded to an average decline of 8.4% and 8.2% in total EE in the VLCD and LCD groups, respectively (Figure 1). Changes in total EE in the fasting group were measured by 24-hr whole-body calorimetry, which showed a daily drop in total EE of -0.34MJ/day and a
cumulative decrease of -1.9MJ/day (p<0.001) after 6 days of fasting (Figure S5 of the Online Supplementary Material). Resting EE remained essentially unchanged after the 6-day fasting period (-0.1MJ/day) whereas a similar, significant decrease (p<0.01) in resting EE was observed in the VLCD and LCD groups after 10%WL (-8.6% and -8.7%, respectively) (Table 3, Figure 1).

3.3 Adaptive Thermogenesis

Adaptive changes in total EE after 10%WL were similar for the VLCD (-1.0MJ/day, -6.2%) and LCD (-1.2MJ/day, -6.8%) groups suggesting a minor influence of rate of WL on the degree of metabolic adaptation (Table 4, Figure 2). Differences between WL groups were more defined for resting EE; 6-day fasting appeared to induce an increase in measured compared to predicted resting EE (+0.4MJ/day) whereas resting EE declined after 10%WL in the LCD (-0.4MJ/day, -5.3%) and VLCD (-0.1MJ/day, -1.4%) groups (Table 4, Figure 2). Adaptive changes in total EE were not associated with rate of WL (n=18, r=0.07, p=0.75, Figure 3a) and weight lost as FFM (ΔFFM/ΔBW; n=18, r=0.26, p=0.27). Adaptive changes in resting EE were significantly associated with rate of WL (n=30, r=-0.42, p=0.01, Figure 3b) and ΔFFM/ΔBW (n=30, r=0.48, p=0.007).

4. Discussion

This study examined for the first time whether three WL diets characterised by different rate of WL had individual effects on resting and total EE in obese men losing a similar amount of body weight. Not surprisingly, the rate of WL was directly associated with negative energy balance and, therefore, was highest during fasting and lowest in the LCD group. However, the difference in WL rates observed during the VLCD and LCD interventions determined a similar decrease in total and resting EE after 10%WL, whereas the 6-day fasting had a minimal effect on resting EE. Hence, the results are not aligned to our initial hypotheses that an accelerated rate of WL was associated with a greater metabolic adaptation.
important interaction was observed between rate of WL and the level of metabolic adaptation for resting EE; specifically, the LCD group was characterised by an adaptive decline in resting EE (-5.3%) whereas a 6-day fasting induced opposite changes in resting EE (i.e., measured resting EE greater than predicted by changes in body composition).

Several studies have reported a significant decline in total and resting EE during WL. Prentice et al. reviewed 29 studies measuring resting EE during WL and reported a decrease in resting EE ranging from 5 to 25%. Two recent systematic reviews of WL studies showed that resting EE declined of 126 kcal/day (0.53MJ/day) after an average WL of 9.4kg. Changes in FM and FFM explained approximately 79% of the variance seen in absolute resting EE post-weight loss. Leibel et al. conducted a seminal study to evaluate the degree of metabolic adaptation in obese and non-obese subjects fed a 800-kcal diet to lose a nominal 10%WL; obese subjects showed a metabolic adaptation of approximately 1.0MJ/day (~9.5%) for total EE and 0.6MJ/day (~7.7%) for resting EE. We found a similar level of metabolic adaptation for total EE in both VLCD (-1.0MJ/day) and LCD (-1.2MJ/day) groups but results for REE were different between the two studies as a lower level of metabolic adaptation was observed in our study. This result could also be partly explained by the different characteristics of the populations such as the inclusion of a greater number of obese women and different methodology for the assessment of body composition changes (i.e., hydrodensitometry) in the study by Leibel et al., which may suggest a dimorphic effect of WL on adaptive thermogenesis. However, given that changes on body composition do not fully explain the variance in EE after weight loss, it has been suggested that regulatory systems of energy stores involving metabolic, neuroendocrine and autonomic responses may be involved. One such regulatory factor is the adipocyte-secreted hormone leptin, which may mediate these adaptive changes in EE. Results from a recent RCT indicate that in addition to leptin, Peptide YY may also be
significantly associated with REE; however FFM, FM and age were the stronger predictors of changes in REE in this study [42].

The effects of different rates of WL in obese subjects has rarely been investigated in controlled studies with repeated measurements of resting and total EE. While some investigators using 24-hr whole-body indirect calorimeters have found that total EE increased or remained stable after WL in post-obese women [3, 43], other studies have observed a decrease [5, 34]. The CALERIE study employed both DLW and 24-hr whole-body calorimetry to measure changes in total EE in overweight subjects randomised to 25% caloric restriction (CR, 6 months) or LCD (~890kcal/day) to induce a 15%WL (3 months WL + 3 months weight maintenance). The results from the 24-hr whole-body calorimetry measurements showed that the amount of total EE not accounted by changes in body composition was similar in the CR (0.5MJ/day) and LCD (-0.5MJ/day) groups despite a different rate of WL between the two groups [5]. Our results are aligned to these data, which seems to indicate that the rate of WL may not influence adaptive changes of total EE [5].

The results obtained from the DLW analyses of total EE in the CALERIE study have been reported in two separate analyses evaluating the effects of different dietary interventions on metabolic adaptation [44, 45]. The first analysis reported a metabolic adaptation of 0.9MJ/day and 1.1MJ/day for the CR and LCD groups, respectively [45]. The second analysis reported the effects of a 12-month CR intervention on total EE in overweight subjects who lost approximately 10.0kg of their initial body weight and reported a metabolic adaptation of approximately 0.8MJ/d (-6.6%) [44]. Our study has observed a similar degree of metabolic adaptation in both VLCD and LCD groups.

At 10% WL, the LCD group showed the greatest loss of FM (~91%) and, although the fasting group had the greatest loss of FFM, this was mainly attributable to the decrease in TBW [21]. Only a few studies have used the 4-compartment model to assess body
composition during VLCD’s in obese patients. Fogelholm et al. \cite{46} assessed fat-mass loss during weight reduction in 32 obese women and found subjects lost 13.2 kg over a 12 week reduction programme, which included 8 weeks of VLCD (2.7 MJ/d and 71g/d protein). On average, the women lost 85% fat mass and 15% FFM. Albu et al. \cite{47} also used the 4-compartment model to assess the composition of WL in 10 obese women who lost 14kg by consuming a 600kcal/d formula and found that FM contributed to 89% of total WL. The present VLCD and LCD groups determined similar proportional changes in FM (range: 80-90%) after 10% WL. Significant amount of WL in humans will always be accompanied by a loss of FFM in addition to FM \cite{48}. However, the relative contributions of FFM and FM to total WL is a function of both the rate and extent of WL, plus the body content of fat prior to WL \cite{48-50}. In the current study, rate of WL had the most pronounced effect on the amount of body fat loss, with the largest losses during the LCD. A slower rate of WL promoted the lowest loss in FFM and determined similar changes in total and resting EE to the VLCD group. Therefore, the LCD strategy would represent the preferred approach to determine the most beneficial changes in body composition and energy expenditure. The present data are novel in this respect and suggest that the energy cost of WL increases as time of dieting proceeds. This has implications for dieters if a steady WL is to be achieved, since a greater negative EB has to be achieved per unit of weight change (kg), reflecting the increasing proportion of fat mass to the change in body weight.

Important limitations of our study were the small sample size and the non-randomized allocation of subjects to the WL interventions. The influence of these two factors was however minimized by 1) the use of state of the art methods for the assessment of energy expenditure (DLW, 24-h whole-body indirect calorimetry and ventilated-hood indirect calorimetry) and body composition (4-compartment model) and 2) the similar phenotypic characteristics of the three groups at baseline. The validity of the results is also strengthened...
by the careful control of energy intake and physical activity, which allowed a detailed
observation of changes in energy balance during the study. We should also mention that the
results of this study are not representative of the entire obese population as our sample
included only men. In addition, the short duration of the fasting group did not allow the
utilisation of DLW to measure total EE; whole-body 24-hr indirect calorimetry was used to
measure total EE for three days but participants entered the calorimeters two days after the
start of the fasting period. We have back-extrapolated the baseline data based on the
assumption of a linear decline in total EE and therefore the interpretation of the total EE
results in this group should be interpreted with caution.

5. Conclusions

An important aim of obesity treatments is to delay WL plateau and prevent weight regain.
Our results show that the amount of FM and FFM lost during WL in obese men was a
function of the level of negative energy balance. The lower rate of WL associated with a
moderate caloric restriction determined a greater FM loss compared to WL interventions
characterised by greater energy deprivation. This may have more beneficial effects on
cardio-metabolic health and, in principle, be more indicated in older subjects to minimize
FFM mobilization during WL. However, differences in WL rate and composition did not
have an independent effect on adaptive changes in total EE after 10%WL, which contributed
to about 6% of the decline in total EE. In conclusion, these results highlight, once again, the
importance of monitoring changes in EE and body composition during WL treatments to
reduce the risk of weight regain and diminish FFM loss by appropriate modifications of
dietary intake and/or physical activity.
Acknowledgments

This work was supported by funding from Scottish Executive and a grant from Slimming World, Alfreton, UK.

Conflict of interest

None to declare

Author contributions

This manuscript was conceived by MS, which was discussed co-written with the other authors (PF, JL, ERG, EM, PR, GEL, ME, JS, AMJ). All authors contributed to subsequent analyses and interpretation. All authors contributed and approved the final revision of the manuscript. The corresponding author (MS) is the guarantor for the manuscript and had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis reported in the manuscript.
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Table 1: Baseline characteristics of participants in the fasting, very low calorie diet (VLCD) and low calorie diet (LCD) groups

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>VLCD</th>
<th>LCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of men</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39 ± 13</td>
<td>46 ± 10</td>
<td>44 ± 7</td>
</tr>
<tr>
<td></td>
<td>(19 – 54)</td>
<td>(28 – 56)</td>
<td>(31–47)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 ± 0.06</td>
<td>1.75 ± 0.05</td>
<td>1.77 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(1.68 – 1.84)</td>
<td>(1.68 – 1.83)</td>
<td>(1.69 – 1.80)</td>
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<tr>
<td>Body weight (kg)</td>
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<td>107.3 ± 15.0</td>
<td>105.6 ± 10.2</td>
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<td>(85.2 – 124.1)</td>
<td>(88.0 – 115.6)</td>
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<tr>
<td>Body Mass Index (kg/m²)</td>
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<td>34.9 ± 3.5</td>
<td>33.7 ± 1.9</td>
</tr>
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<td></td>
<td>(31.0 – 38.5)</td>
<td>(30.3 – 39.5)</td>
<td>(30.8 – 36.1)</td>
</tr>
<tr>
<td>Body fat (% of body weight)</td>
<td>36.1 ± 3.6</td>
<td>41.9 ± 4.2</td>
<td>38.3 ± 5.0</td>
</tr>
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<td></td>
<td>(32.4 – 41.6)</td>
<td>(38.2 – 47.9)</td>
<td>(32.6 – 44.7)</td>
</tr>
</tbody>
</table>

Data are mean ± SD (range). Baseline values were not statistically different between groups.
Table 2: Changes in body composition in obese men during fasting, very low calorie diet (VLCD) and low calorie diet (LCD)

<table>
<thead>
<tr>
<th></th>
<th>Body weight (kg)</th>
<th>∆ (kg)</th>
<th>FFM (kg)</th>
<th>∆ (kg)</th>
<th>FM (kg)</th>
<th>∆ (kg)</th>
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<tr>
<td><strong>Fasting (n=6)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>107.1±11.5</td>
<td></td>
<td>68.4±7.1</td>
<td></td>
<td>38.7±6.5</td>
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<tr>
<td>5%WL</td>
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<td>-6.0±1.3#</td>
<td>65.6±7.7</td>
<td>-2.8±0.6#</td>
<td>35.5±6.3</td>
<td>-3.2±0.5#, a</td>
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<tr>
<td><strong>VLCD (n=6)</strong></td>
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<td>-3.6±1.0#</td>
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<tr>
<td>10%WL</td>
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<td>-9.2±1.2#, b</td>
<td>60.3±6.8</td>
<td>-1.7±0.6#</td>
<td>37.9±9.5</td>
<td>-7.4±0.8#, b</td>
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<tr>
<td><strong>LCD (n=6)</strong></td>
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<tr>
<td>Baseline</td>
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<td>64.6±3.6</td>
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<td>40.8±8.7</td>
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<tr>
<td>5%WL</td>
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<td>63.4±3.3</td>
<td>-1.2±1.0</td>
<td>34.9±8.8</td>
<td>-5.9±1.4#</td>
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<tr>
<td>10%WL</td>
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<td>63.5±3.8</td>
<td>-1.1±1.1</td>
<td>29.3±9.4</td>
<td>-11.5±2.0#</td>
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</table>

Data for the absolute values are shown as mean±SD. Changes (Δ) relative to baseline are reported. FFM = fat free mass; FM= fat mass. 5%WL is nominal 5% weight loss relative to baseline. 10%WL is nominal 10% weight loss relative to baseline. *: statistically significant compared to baseline within each WL group (paired t-test, p<0.01); between-intervention comparison of changes (Δ) in body weight, FFM and FM (independent t-test): a p<0.01: fasting 5%WL vs LCD 5%WL; b p<0.01: LCD 10%WL vs VLCD 10%WL.
Table 3: Changes in total (TEE) and resting energy expenditure (REE) during fasting, very low calorie diet (VLCD) and low calorie diet (LCD)

<table>
<thead>
<tr>
<th></th>
<th>TEE (MJ/day)</th>
<th></th>
<th>REE (MJ/day)</th>
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<tbody>
<tr>
<td></td>
<td>DLW</td>
<td>Δ</td>
<td>IC</td>
<td>Δ</td>
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</tr>
<tr>
<td>Baseline</td>
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</tr>
<tr>
<td>5% WL</td>
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</tr>
<tr>
<td>10% WL</td>
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</tr>
<tr>
<td>VLCD (n=6)</td>
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<tr>
<td>Baseline</td>
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</tr>
<tr>
<td>5% WL</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10% WL</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LCD (n=6)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
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<td></td>
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</tr>
<tr>
<td>5% WL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% WL</td>
<td></td>
<td></td>
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</tbody>
</table>

Data for the absolute values are shown as mean±SD. Changes (Δ) relative to baseline are reported. IC= indirect calorimetry. 5%WL is nominal 5% weight loss relative to baseline. 10%WL is nominal 10% weight loss relative to baseline. #: statistically significant compared to baseline within each WL group; Data for the absolute values are shown as mean±SD. FFM = fat free mass; FM= fat mass. 5%WL is nominal 5% weight loss relative to baseline. 10%WL is nominal 10% weight loss relative to baseline. #: statistically significant compared to baseline within each WL group (paired t-test, p<0.01). Differences in TEE and REE between WL interventions were not significant.
Table 4: Differences between the measured and predicted total (TEE) and resting (REE) energy expenditure at the end of each phase were calculated to assess metabolic adaptation after weight loss (5% and 10%WL) in the fasting, very low calorie diet (VLCD), and low calorie diet (LCD) groups.

<table>
<thead>
<tr>
<th>TEE &lt;sub&gt;DLW&lt;/sub&gt;</th>
<th>REE</th>
<th>MJ/day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%WL</td>
<td></td>
<td>+0.4±0.6</td>
</tr>
<tr>
<td><strong>VLCD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%WL</td>
<td></td>
<td>+0.2±0.4</td>
</tr>
<tr>
<td>10%WL</td>
<td></td>
<td>-0.1±0.4</td>
</tr>
<tr>
<td><strong>LCD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%WL</td>
<td></td>
<td>+0.3±1.5</td>
</tr>
<tr>
<td>10%WL</td>
<td></td>
<td>-0.2±0.4</td>
</tr>
</tbody>
</table>

Data are shown as mean±SD. TEE <sub>DLW</sub> = TEE measured by doubly labelled water; Predicted REE and TEE were estimated by using prediction equations which were developed using the baseline data for TEE <sub>DLW</sub> (N = 12) and REE (N = 18). Multiple linear regression analysis was used to predict REE and TEE. REE or TEE were entered as dependent variables and fat mass (FM, kg) and fat free mass (FFM, kg) were the independent variables. The equations derived and used for the prediction of REE and TEE were: REE (MJ/day): 1.44+(0.078*FFM)+(0.039*FM) $R^2$=0.62, F=12.29, p<0.01; TEE <sub>DLW</sub> (MJ/day)= -5.66+(0.38*FFM)-(0.05*FM), $R^2$=0.70, F=10.3, p=0.005. Baseline predicted RMR and TEE were not significantly different from measured values (paired t test; REE: 0.04±0.5MJ/day, p=0.73; TEE <sub>DLW</sub>: 0.2±1.3MJ/day, p=0.52). These equations were then used to estimate REE and TEE in each WL group. The results are based on the absolute difference between the measured and predicted REE and TEE at the end of each phase. Negative values greater indicate that changes in body composition did not account for the observed changes in energy expenditure suggesting the existence of adaptive energetic mechanisms. Differences in TEE and REE between WL interventions were not significant.
Figure Legends

Figure 1: Mean percent changes in total (TEE) and resting energy expenditure (REE) after 5% weight loss (WL) during fasting and 10% WL during very low calorie diet (VLCD) and low calorie diet (LCD). TEE was measured by doubly labelled water (TEE_DLW). Error bars are 95%CI. Changes in TEE and REE were not statistically different between WL interventions reaching a similar WL target (5%WL, 10%WL). Independent t-test was used to test differences between WL groups.

Figure 2: Metabolic Adaptation - Percent of total and resting energy expenditure not accounted by changes in body composition (FFM and FM) after 5% and 10% weight loss (WL) in obese assigned to three different WL interventions: fasting, very low calorie diet (VLCD) and low calorie diet (LCD). TEE was measured by doubly labelled water (DLW). Bar charts are: mean±95%CI. The extent of metabolic adaptation was not statistically different (p>0.05) between WL interventions reaching a similar WL target (5%WL, 10%WL). Independent t-test was used to test differences between WL groups.

Figure 3: Association between rate of weight loss (WL) with adaptive changes in total (TEE, 3a) and resting (REE, 3b) energy expenditure in obese men. TEE was measured by doubly labelled water; therefore data were only available for the VLCD and LCD groups. Measurements of REE were also available for the fasting group which determined the greater sample size observed in this analysis. r= Pearson’s coefficient of correlation.
n=18, r=0.07, p=0.75
Response to Reviewers’ Comments

Reviewers’ comments:

Your paper is now acceptable for publication but I thought of giving you one more opportunity to check the following before final acceptance is offered. Metabolism has implemented a new set of guidelines for authors. Please refer to these guidelines at http://www.metabolismjournal.com/authorinfo and format your manuscript accordingly. Only manuscripts that are in the proper format are considered.

Reply: Thank you. Our manuscript has been revised to adhere to the journal guidelines.

Please also perform an updated literature search and cite any relevant papers recently published in Metabolism or elsewhere. Consider whether you would like to add a few words on the possible role of leptin in the changes observed after weight loss. Not necessary but you may want to do so to enhance the spectrum of discussion.

Reply: Thank you. New relevant references have been added to our manuscript. In addition we have now briefly added a paragraph on a possible role for leptin in the observed findings.

Please scrutinize statistics, data presentation and more specifically tables and graphs. Please remove lines from tables unless absolutely necessary. Please make sure all tables and all legends of figures present units of variables, n of subjects, explanation of symbols used in graphs and all other information needed by the authors to easily understand your message. Consider showing stat. significance with symbols as needed and explain in legends what the symbols mean as well as what error bars mean. Please remove upper and right perpendicular lines from the frame of graphs.

Reply: Thank you. We have now addressed all the issues raised and hope that our manuscript is formatted according to the Journal requirements.
Supplementary Material
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