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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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Elsevier Editorial System(tm) for Metabolism
Manuscript Draft

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

1 **IMPOSED RATE AND EXTENT OF WEIGHT LOSS IN OBESE MEN**
2 **AND ADAPTIVE CHANGES IN RESTING AND TOTAL ENERGY**
3 **EXPENDITURE**

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

20

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22

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

25

26 The authors have no conflicts of interest to declare

27

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

29

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

34

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

36

37 **Abstract**

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

43 Methods: Three groups of six, obese men participated in a total fast for 6 days to achieve a
44 5% WL and a very low calorie (VLCD, 2.5MJ/day) for 3 weeks or a low calorie (LCD,
45 5.2MJ/day) diet for 6 weeks to achieve a 10% WL. A four-component model was used to
46 measure body composition. Indirect calorimetry was used to measure resting EE. Total EE
47 was measured by doubly labelled water (VLCD, LCD) and 24-hr whole-body calorimetry
48 (fasting).

49 Results: VLCD and LCD showed a similar degree of metabolic adaptation for total EE
50 (VLCD=-6.2%; LCD=-6.8%). Metabolic adaptation for resting EE was greater in the LCD
51 (-0.4MJ/day, -5.3%) compared to the VLCD (-0.1MJ/day, -1.4%) group. Resting EE did not
52 decrease after short-term fasting and no evidence of adaptive thermogenesis (+0.4MJ/day)
53 was found after 5% WL. The rate of WL was inversely associated with changes in resting EE
54 (n=30, r=0.-42, p=0.01).

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

58

59 1. **Introduction**

60 Weight loss (WL) is associated with modifications of fuel oxidation and resting and total
61 energy expenditure (EE)[1, 2]. Changes in body composition [i.e., fat mass (FM) and fat free
62 mass (FFM)] explain a large proportion of the decrease in EE, which may be linked to the
63 loss of metabolically active cellular mass, lower dietary induced thermogenesis (DIT) and
64 energy cost of physical activity[1, 2]. The residual EE not accounted for by the observed
65 body composition and metabolic changes could derive from modifications of the efficiency
66 and activity of metabolic, endocrine and autonomic pathways (i.e., adaptive
67 thermogenesis)[1, 2].

68 However, the occurrence of adaptive metabolic changes during WL is not a consistent
69 finding across WL studies[3-8]. These differences could be explained by the different
70 approaches used to quantify metabolic adaptation, such as application of different for the
71 measurement of body composition and/or energy expenditure, as well as to the
72 characteristics of the study population (adiposity, age, health status), degree of negative
73 energy balance and duration of the WL interventions[1]. Specifically, the level of the
74 negative energy balance (i.e., very low calorie diet (VLCD), low calorie diet (LCD)), the
75 macronutrient composition of the hypocaloric diets (i.e., high protein, low fat) and the type
76 (i.e., resistance, aerobic) and intensity (i.e., workload and frequency) of physical activity can
77 influence the rate of weight change (how quickly you lose weight over time, kg/d), and
78 amount of WL (total loss in kg, or relative loss %)[1, 5, 9-12].

79 Several studies have investigated the effects of fasting or energy-restricted diets on body
80 composition and EE in obese subjects [6, 13-18]. These studies aimed primarily at testing the
81 effects of the extent of WL on EE; however, none of them has so far compared the effects of
82 diets inducing different rates of WL on resting and total EE in controlled, experimental
83 settings. The majority of these studies have been conducted in free-living conditions, which

84 may have contributed to the inconsistent results and, consequently, fuelled the debate on the
85 existence and physiological relevance of adaptive thermogenesis associated with WL[19,
86 20].

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

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

97 2. **Materials and methods**

98 2.1 Subject characteristics

99 Eighteen (n=6 in each group), healthy, non-smoking, obese (body mass index (BMI) = 33-40
100 kg/m²) male subjects, aged between 19-55 years, were recruited. Subjects were not following
101 any special diet and were not prescribed any regular medication. A description of the
102 inclusion and exclusion criteria is reported in the **Online Supplementary Material**. The
103 study was approved by the Grampian Research Ethics Committee. Written informed consent
104 was obtained.

105 2.2 Experimental design

106 Subjects were non-randomly allocated to three WL interventions (fasting, VLCD, LCD) with
107 a similar study design as previously described[21]. A description of the study protocol for
108 each WL intervention is provided in the online supplementary material (**Figure S1-S3**).

109 Briefly, during the 6-day baseline period subjects consumed a fixed maintenance diet (13%
110 protein, 30% fat and 57% carbohydrate). After the 7-day baseline period, each group
111 followed the specific diet to lose 5% and 10% of their baseline body weight. However, the
112 duration of the fasting was of 6 days as ethical constraint allowed to fast subjects to lose 5%
113 of their baseline body weight. The duration of the WL phases to achieve a 10% WL was of 3
114 and 6 weeks for the VLCD and LCD groups, respectively. Throughout the study, participants
115 were residential in the Human Nutrition Unit at the Rowett Institute of Nutrition and Health
116 (RINH), Aberdeen, UK. All food and drinks consumed by each participant during the study
117 were supplied by the dietetics staff in the Unit. The participants were requested not to
118 undertake any other strenuous physical activity during the study and they were asked to
119 record their individual exercise sessions.

120 2.3 Energy and dietary intake

121 Energy intake (EI) was measured daily, based on the recorded weighed intakes of food and
122 drink and using values from McCance and Widdowson, 'The composition of foods'[22].
123 During starvation, the participants had access to water only. The VLCD comprised: daily
124 weight 642g, energy 2.55kJ/g, protein 49.4g (32%), carbohydrate 52.8g (35%), and fat 23.1g
125 (33%). The LCD comprised: daily weight 1260g, energy 5.2kJ/g, protein 50.3g (17%),
126 carbohydrate 155.7g (50%), and fat 45.4g (33%). Further details are provided in **Table S1 of**
127 **the Online Supplementary Material**. Diets and recipes are available upon request. The
128 Department of Health and Social Security (1987) guidelines were adopted for the design of
129 the WL diets and ensure a balanced intake of protein, minerals and vitamins[23].

130 2.4 Resting Energy Expenditure

131 REE was measured at baseline and at the end of each WL phase (5% and 10% WL) by
132 indirect calorimetry over 30–40 min using a ventilated hood system (Deltatrac II, MBM-200,
133 Datex Instrumentarium Corporation, Finland). During the measurement, subjects lay on a bed

134 in a thermo-neutral room and were instructed to lie still but not to fall asleep. Resting EE
135 was calculated from minute-by-minute data using the mean of 15 min of stable
136 measurements, with the first and last 5 min excluded. The equations of Elia and Livesey[24]
137 were used to derive resting EE. Details of calibration burns and repeatability testing have
138 been described previously[25].

139 2.5 Total Energy expenditure

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

154 Measurement of total EE by doubly labelled water technique: Subjects in both VLCD and
155 LCD groups were dosed orally with doubly labelled water on the morning of day 7. Subjects
156 received a bolus dose of DLW to estimate total EE during the following 10-day period. At
157 07:00 hours, subjects were woken up and asked to empty their bladders and were weighed.
158 At 09:00 hours, they provided a urine sample to be used as baseline, along with two further

159 background samples to provide information on the pre-dose isotopic enrichment of the
160 subject's body water pools. Immediately after providing the 09:00 hour sample, each subject
161 was asked to consume a pre-prepared dose of $^2\text{H}_2^{18}\text{O}$. The dose, bottle and straw used for
162 dose consumption were weighed before and after dosing to two decimal places to allow for
163 accurate determination of the quantity consumed by the subject. Subjects also consumed 100
164 ml of tap water after the dose to prevent isotope loss from the subject's buccal cavity. The
165 dose levels were: 0.15g $^2\text{H}_2\text{O}$ /kg body weight and 0.9g H_2^{18}O /kg body weight. Each dose
166 was prepared, sealed and autoclaved the day before dosing. Subjects then collected aliquots
167 of urine at 4, 5 and 6 hours after dosing to enable plateau to be individually measured using
168 the "slope intercept" method. Subjects continued to collect an aliquot sample at 11.00 hours
169 for the next 10 days. Subjects in the VLCD groups received 2 doses over a period of 20 days
170 (day 7 and day 18). Subjects in the LCD groups received 4 doses over a period of 40 days
171 (day 7, day 18, Day 28, day 39). Samples were immediately frozen at -20°C after collection.
172 Urine samples were collected for a multi-point stable-isotope analysis using gas isotope ratio
173 mass-spectrometry. Urine isotope enrichments were determined using the platinum
174 equilibration technique[28] for ^2H and the CO_2 equilibration technique[29] for ^{18}O . The log-
175 transformed data of enrichment by time was extrapolated back to time zero, giving a
176 theoretical enrichment at time zero, which provided information on the individual's size of
177 the body water pool assuming the dilution principle. Isotopic enrichment of the post-dose
178 urine samples was analysed relative to the original background amounts. Isotope turnover
179 rates, water pool sizes and CO_2 production were calculated using the multipoint method [30].
180 Total EE was calculated from CO_2 production using classical respirometry formulae and
181 measured food quotient from the provided diets.

182 2.6 Measurement of body composition

183 Subject height was measured to the nearest 0.1 cm (Holtain Ltd. Crymych, Dyfeld, Wales,
184 UK) and body weight was measured each morning to the nearest 50 g (DIGI DS-410, CMS
185 Weighing Equipment London, UK). The four-compartment model of body composition as
186 described by Fuller et al[31] was used to measure FM and FFM. Total body water (TBW-kg)
187 was measured by deuterium dilution (D_2O) as described by Pullicino et al[32]. Bone mineral
188 mass (BMM-kg) was measured by dual energy X-ray absorptiometry scanning (DEXA;
189 Norland XR-26, Norland corporation, Wisconsin, USA). Body volume and density was
190 measured using a system of air displacement (Bod Pod, Life Measurement Instruments,
191 Concord, Connecticut, USA). The measurement protocols of the various body composition
192 methods have been previously reported[21].

193 2.7 Statistical analysis

194 Data are reported as mean and standard deviation. Error bars in the figures are standard error
195 of means. Multiple linear regression analysis was performed on the pooled baseline data
196 from the three WL interventions (N = 18) to derive sample-specific prediction equations for
197 the estimation of resting and total EE. Resting and total EE were entered as the dependent
198 variables and fat mass (FM, kg) and fat free mass (FFM, kg) were the independent variables.
199 These equations were then used to predict resting and total EE at the end of the weight loss
200 (5% WL, 10% WL) interventions. Absolute and relative differences between the measured
201 and predicted resting and total EE were calculated for each WL group to evaluate the
202 presence of adaptive thermogenesis. Paired t-test was used to test whether within-subject
203 changes in body composition and EE in each WL intervention. Independent t-test was used
204 to test differences between WL interventions. Subjects in the fasting group resided in the 24-
205 hr whole-body indirect calorimetry for 3 days (Day 3, Day 4, Day 5). A linear regression
206 model was fitted to the 3-day calorimetry data to impute the remaining missing data
207 (Baseline, Day 1, Day 2, Day 6). This approach was based on the assumption of a linear

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

215 3. Results

216 3.1 Weight loss and changes in body composition

217 Baseline characteristics of subjects included in the three WL groups are reported in **Table 1**.
218 Weight loss in the fasting group was 6.0 kg over 6 days. The VLCD group lost 5.2 and 9.2kg
219 over 11 and 21 days and the LCD group lost 7.2 and 12.6 kg over 21 and 42 days,
220 respectively. Mean rates of WL during the 5% WL period were different between the fasting
221 (-1.01 kg/d), VLCD (-0.52 kg/d) and LCD (-0.35 kg/d) groups. The LCD groups lost more
222 FM after each WL phase compared to the fasting ($p < 0.01$) and VLCD ($p < 0.01$) groups
223 (**Table 2**). The fraction of FFM to total WL after 5%WL was 46, 30 and 18% for the fasting,
224 VLCD and LCD groups respectively. At 10% WL, the VLCD losses were 20% FFM and
225 80% FM compared with 9% FFM and 91% FM in the LCD group (**Figure S4 of the Online**
226 **Supplementary Material**).

227 3.2 Changes in total and resting energy expenditure

228 Changes in total EE measured by DLW at the end of the 10% WL phase were not different
229 between the VLCD (-1.3MJ/day) and LCD (-1.5MJ/day) groups ($p > 0.05$). This corresponded
230 to an average decline of 8.4% and 8.2% in total EE in the VLCD and LCD groups,
231 respectively (**Figure 1**). Changes in total EE in the fasting group were measured by 24-hr
232 whole-body calorimetry, which showed a daily drop in total EE of -0.34MJ/day and a

233 cumulative decrease of -1.9MJ/day ($p<0.001$) after 6 days of fasting (**Figure S5 of the**
234 **Online Supplementary Material**). Resting EE remained essentially unchanged after the 6-
235 day fasting period (-0.1MJ/day) whereas a similar, significant decrease ($p<0.01$) in resting
236 EE was observed in the VLCD and LCD groups after 10%WL (-8.6% and -8.7%,
237 respectively) (**Table 3, Figure 1**).

238 3.3 Adaptive Thermogenesis

239 Adaptive changes in total EE after 10%WL were similar for the VLCD (-1.0MJ/day, -6.2%)
240 and LCD (-1.2MJ/day, -6.8%) groups suggesting a minor influence of rate of WL on the
241 degree of metabolic adaptation (**Table 4, Figure 2**). Differences between WL groups were
242 more defined for resting EE; 6-day fasting appeared to induce an increase in measured
243 compared to predicted resting EE (+0.4MJ/day) whereas resting EE declined after 10%WL in
244 the LCD (-0.4MJ/day, -5.3%) and VLCD (-0.1MJ/day, -1.4%) groups (**Table 4, Figure 2**).
245 Adaptive changes in total EE were not associated with rate of WL ($n=18$, $r=0.07$, $p=0.75$,
246 **Figure 3a**) and weight lost as FFM ($\Delta\text{FFM}/\Delta\text{BW}$; $n=18$, $r=0.26$, $p=0.27$). Adaptive changes
247 in resting EE were significantly associated with rate of WL ($n=30$, $r=-0.42$, $p=0.01$, **Figure**
248 **3b**) and $\Delta\text{FFM}/\Delta\text{BW}$ ($n=30$, $r=0.48$, $p=0.007$).

249 4. Discussion

250 This study examined for the first time whether three WL diets characterised by different rate
251 of WL had individual effects on resting and total EE in obese men losing a similar amount of
252 body weight. Not surprisingly, the rate of WL was directly associated with negative energy
253 balance and, therefore, was highest during fasting and lowest in the LCD group. However,
254 the difference in WL rates observed during the VLCD and LCD interventions determined a
255 similar decrease in total and resting EE after 10%WL, whereas the 6-day fasting had a
256 minimal effect on resting EE. Hence, the results are not aligned to our initial hypotheses that
257 an accelerated rate of WL was associated with a greater metabolic adaptation. However, an

258 important interaction was observed between rate of WL and the level of metabolic adaptation
259 for resting EE; specifically, the LCD group was characterised by an adaptive decline in
260 resting EE (-5.3%) whereas a 6-day fasting induced opposite changes in resting EE (i.e.,
261 measured resting EE greater than predicted by changes in body composition).

262 Several studies have reported a significant decline in total and resting EE during WL[17, 33,
263 34] [7, 35, 36]. Prentice et al.[37] reviewed 29 studies measuring resting EE during WL and
264 reported a decrease in resting EE ranging from 5 to 25%. Two recent systematic reviews of
265 WL studies showed that resting EE declined of 126 kcal/day (0.53MJ/day) after an average
266 WL of 9.4kg. Changes in FM and FFM explained approximately 79% of the variance seen in
267 absolute resting EE post-weight loss [38, 39]. Leibel et al[7] conducted a seminal study to
268 evaluate the degree of metabolic adaptation in obese and non-obese subjects fed a 800-kcal
269 diet to lose a nominal 10% WL; obese subjects showed a metabolic adaptation of
270 approximately 1.0MJ/day (~9.5%) for total EE and 0.6MJ/day (~7.7%) for resting EE. We
271 found a similar level of metabolic adaptation for total EE in both VLCD (-1.0MJ/day) and
272 LCD (-1.2MJ/day) groups but results for REE were different between the two studies as a
273 lower level of metabolic adaptation was observed in our study. This result could also be
274 partly explained by the different characteristics of the populations such as the inclusion of a
275 greater number of obese women and different methodology for the assessment of body
276 composition changes (i.e., hydrodensitometry) in the study by Leibel et al[7], which may
277 suggest a dimorphic effect of WL on adaptive thermogenesis. However, given that changes
278 on body composition do not fully explain the variance in EE after weight loss, it has been
279 suggested that regulatory systems of energy stores involving metabolic, neuroendocrine and
280 autonomic responses may be involved [2]. One such regulatory factor is the adipocyte-
281 secreted hormone leptin [40], which may mediate these adaptive changes in EE [2, 41].
282 Results from a recent RCT indicate that in addition to leptin, Peptide YY may also be

283 significantly associated with REE; however FFM, FM and age were the stronger predictors
284 of changes in REE in this study [42].

285 The effects of different rates of WL in obese subjects has rarely been investigated in
286 controlled studies with repeated measurements of resting and total EE. While some
287 investigators using 24-hr whole-body indirect calorimeters have found that total EE
288 increased or remained stable after WL in post-obese women[3, 43], other studies have
289 observed a decrease[5, 34]. The CALERIE study employed both DLW and 24-hr whole-
290 body calorimetry to measure changes in total EE in overweight subjects randomised to 25%
291 caloric restriction (CR, 6 months) or LCD (~890kcal/day) to induce a 15% WL (3 months
292 WL + 3 months weight maintenance). The results from the 24-hr whole-body calorimetry
293 measurements showed that the amount of total EE not accounted by changes in body
294 composition was similar in the CR (0.5MJ/day) and LCD (-0.5MJ/day) groups despite a
295 different rate of WL between the two groups[5]. Our results are aligned to these data, which
296 seems to indicate that the rate of WL may not influence adaptive changes of total EE[5].
297 The results obtained from the DLW analyses of total EE in the CALERIE study have been
298 reported in two separate analyses evaluating the effects of different dietary interventions on
299 metabolic adaptation[44, 45]. The first analysis reported a metabolic adaptation of 0.9MJ/day
300 and 1.1MJ/day for the CR and LCD groups, respectively[45]. The second analysis reported
301 the effects of a 12-month CR intervention on total EE in overweight subjects who lost
302 approximately 10.0kg of their initial body weight and reported a metabolic adaptation of
303 approximately 0.8MJ/d (-6.6%)[44]. Our study has observed a similar degree of metabolic
304 adaptation in both VLCD and LCD groups.

305 At 10% WL, the LCD group showed the greatest loss of FM (~91%) and, although the
306 fasting group had the greatest loss of FFM, this was mainly attributable to the decrease in
307 TBW[21]. Only a few studies have used the 4-compartment model to assess body

308 composition during VLCD's in obese patients. Fogelholm et al. [46] assessed fat-mass loss
309 during weight reduction in 32 obese women and found subjects lost 13.2 kg over a 12 week
310 reduction programme, which included 8 weeks of VLCD (2.7 MJ/d and 71g/d protein). On
311 average, the women lost 85% fat mass and 15% FFM. Albu et al.[47] also used the 4-
312 compartment model to assess the composition of WL in 10 obese women who lost 14kg by
313 consuming a 600kcal/d formula and found that FM contributed to 89% of total WL. The
314 present VLCD and LCD groups determined similar proportional changes in FM (range: 80-
315 90%) after 10% WL. Significant amount of WL in humans will always be accompanied by a
316 loss of FFM in addition to FM [48]. However, the relative contributions of FFM and FM to
317 total WL is a function of both the rate and extent of WL, plus the body content of fat prior to
318 WL[48-50]. In the current study, rate of WL had the most pronounced effect on the amount
319 of body fat loss, with the largest losses during the LCD. A slower rate of WL promoted the
320 lowest loss in FFM and determined similar changes in total and resting EE to the VLCD
321 group. Therefore, the LCD strategy would represent the preferred approach to determine the
322 most beneficial changes in body composition and energy expenditure. The present data are
323 novel in this respect and suggest that the energy cost of WL increases as time of dieting
324 proceeds. This has implications for dieters if a steady WL is to be achieved, since a greater
325 negative EB has to be achieved per unit of weight change (kg), reflecting the increasing
326 proportion of fat mass to the change in body weight.

327 Important limitations of our study were the small sample size and the non-randomized
328 allocation of subjects to the WL interventions. The influence of these two factors was
329 however minimized by 1) the use of state of the art methods for the assessment of energy
330 expenditure (DLW, 24-h whole-body indirect calorimetry and ventilated-hood indirect
331 calorimetry) and body composition (4-compartment model) and 2) the similar phenotypic
332 characteristics of the three groups at baseline. The validity of the results is also strengthened

333 by the careful control of energy intake and physical activity, which allowed a detailed
334 observation of changes in energy balance during the study. We should also mention that the
335 results of this study are not representative of the entire obese population as our sample
336 included only men. In addition, the short duration of the fasting group did not allow the
337 utilisation of DLW to measure total EE; whole-body 24-hr indirect calorimetry was used to
338 measure total EE for three days but participants entered the calorimeters two days after the
339 start of the fasting period. We have back-extrapolated the baseline data based on the
340 assumption of a linear decline in total EE and therefore the interpretation of the total EE
341 results in this group should be interpreted with caution.

342 5. **Conclusions**

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

355

356 **Acknowledgments**

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

359

360 **Conflict of interest**

361 None to declare

362

363 **Author contributions**

364 This manuscript was conceived by MS, which was discussed co-written with the other
365 authors (PF, JL, ERG, EM, PR, GEL, ME, JS, AMJ). All authors contributed to subsequent
366 analyses and interpretation. All authors contributed and approved the final revision of the
367 manuscript. The corresponding author (MS) is the guarantor for the manuscript and had full
368 access to all of the data in the study and takes responsibility for the integrity of the data and
369 the accuracy of the data analysis reported in the manuscript.

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502

Table 1: Baseline characteristics of participants in the fasting, very low calorie diet (VLCD) and low calorie diet (LCD) groups

	Fasting	VLCD	LCD
Number of men	6	6	6
Age (years)	39 ± 13 (19 – 54)	46 ± 10 (28 – 56)	44 ± 7 (31-47)
Height (m)	1.76 ± 0.06 (1.68 – 1.84)	1.75 ± 0.05 (1.68 – 1.83)	1.77 ± 0.04 (1.69 – 1.80)
Body weight (kg)	107.2 ± 11.5 (93.8 – 123.5)	107.3 ± 15.0 (85.2 – 124.1)	105.6 ± 10.2 (88.0 – 115.6)
Body Mass Index (kg/m²)	34.7 ± 2.5 (31.0 – 38.5)	34.9 ± 3.5 (30.3 – 39.5)	33.7 ± 1.9 (30.8 – 36.1)
Body fat (% of body weight)	36.1 ± 3.6 (32.4 – 41.6)	41.9 ± 4.2 (38.2 – 47.9)	38.3 ± 5.0 (32.6 – 44.7)

Data are mean ± SD (range). Baseline values were not statistically different between groups.

503
504

Table 2: Changes in body composition in obese men during fasting, very low calorie diet (VLCD) and low calorie diet (LCD)

	Body weight (kg)	Δ (kg)	FFM (kg)	Δ (kg)	FM (kg)	Δ (kg)
Fasting (n=6)						
Baseline	107.1±11.5		68.4±7.1		38.7±6.5	
5% WL	101.1±12.2	-6.0±1.3 [#]	65.6±7.7	-2.8±0.6 [#]	35.5±6.3	-3.2±0.5 ^{#, a}
VLCD (n=6)						
Baseline	107.3±14.9		62.0±6.9		45.3±9.9	
5% WL	102.1±14.3	-5.2±0.8 [#]	60.4±7.1	-1.5±0.4 [#]	41.7±9.6	-3.6±1.0 [#]
10% WL	98.1±14.1	-9.2±1.2 ^{#, b}	60.3±6.8	-1.7±0.6 [#]	37.9±9.5	-7.4±0.8 ^{#, b}
LCD (n=6)						
Baseline	105.4±10.0		64.6±3.6		40.8±8.7	
5% WL	98.2±9.9	-7.2±1.5 [#]	63.4±3.3	-1.2±1.0	34.9±8.8	-5.9±1.4 [#]
10% WL	92.8±9.9	-12.6±2.4 [#]	63.5±3.8	-1.1±1.1	29.3±9.4	-11.5±2.0 [#]

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)

	TEE (MJ/day)		REE (MJ/day)	
	DLW	Δ	IC	Δ
Fasting (n=6)				
Baseline			8.4±1.0	
5%WL			8.3±1.0	-0.1±0.1
VLCD (n=6)				
Baseline	15.6±2.3		8.2±0.7	
5%WL			7.9±0.9	-0.3±0.2
10%WL	14.3±2.4	-1.3±1.4	7.5±0.8	-0.7±0.3 [#]
LCD (n=6)				
Baseline	17.3±2.3		7.8±0.5	
5%WL	17.0±1.3	-0.3±1.7	7.5±0.5	-0.3±0.1 [#]
10%WL	15.8±2.1	-1.5±1.7	7.1±0.3	-0.7±0.4 [#]

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.

	TEE_{DLW}	REE
		MJ/day
Fasting		
5% WL		+0.4±0.6
VLCD		
5% WL		+0.2±0.4
10% WL	-1.0±1.6	-0.1±0.4
LCD		
5% WL	+0.3±1.5	-0.2±0.4
10% WL	-1.2±2.1	-0.4±0.4

Data are shown as mean±SD. TEE_{DLW} = 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_{DLW} (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_{DLW} (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: $0.04±0.5$ MJ/day, $p=0.73$; TEE_{DLW}: $0.2±1.3$ MJ/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.

Figure
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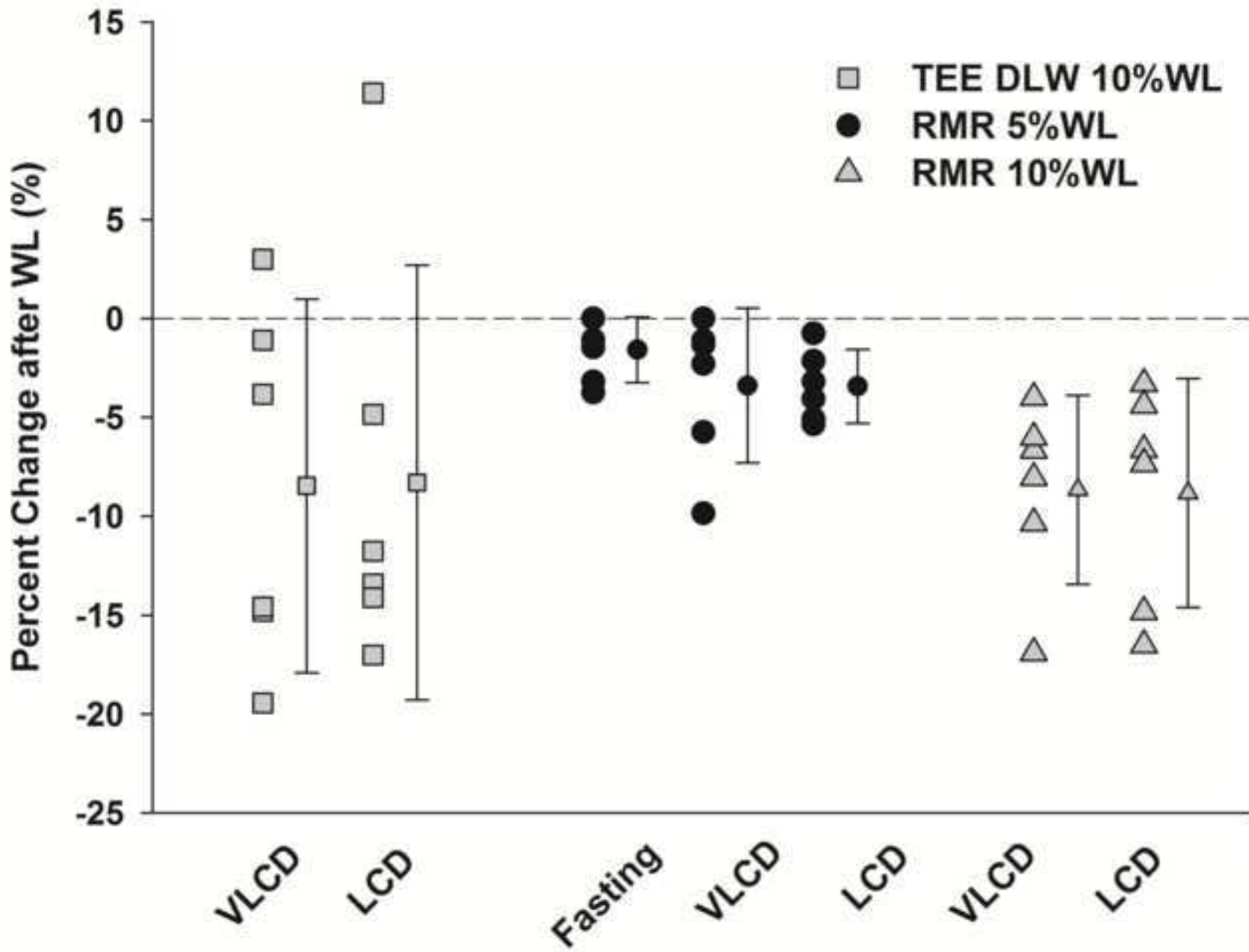
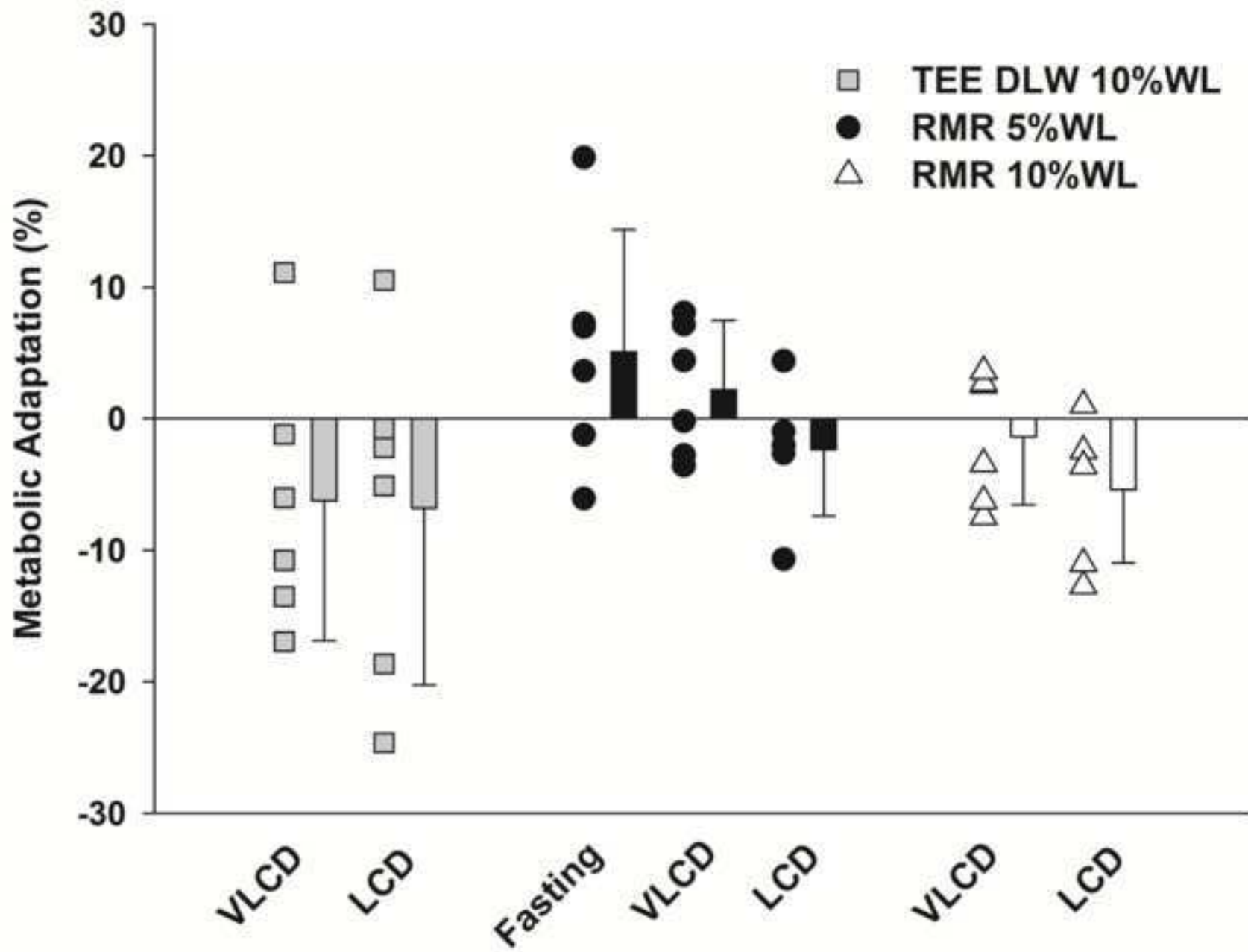
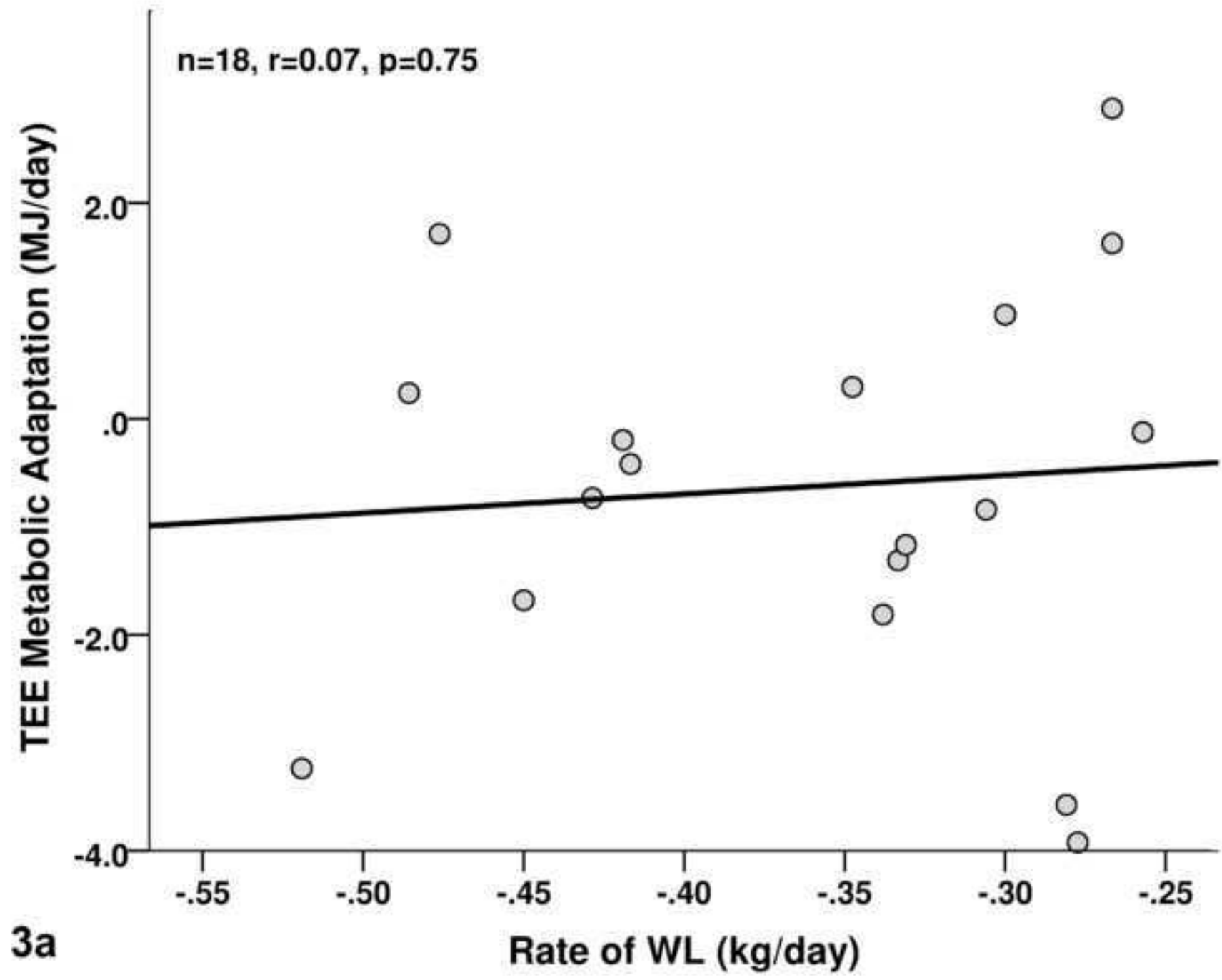
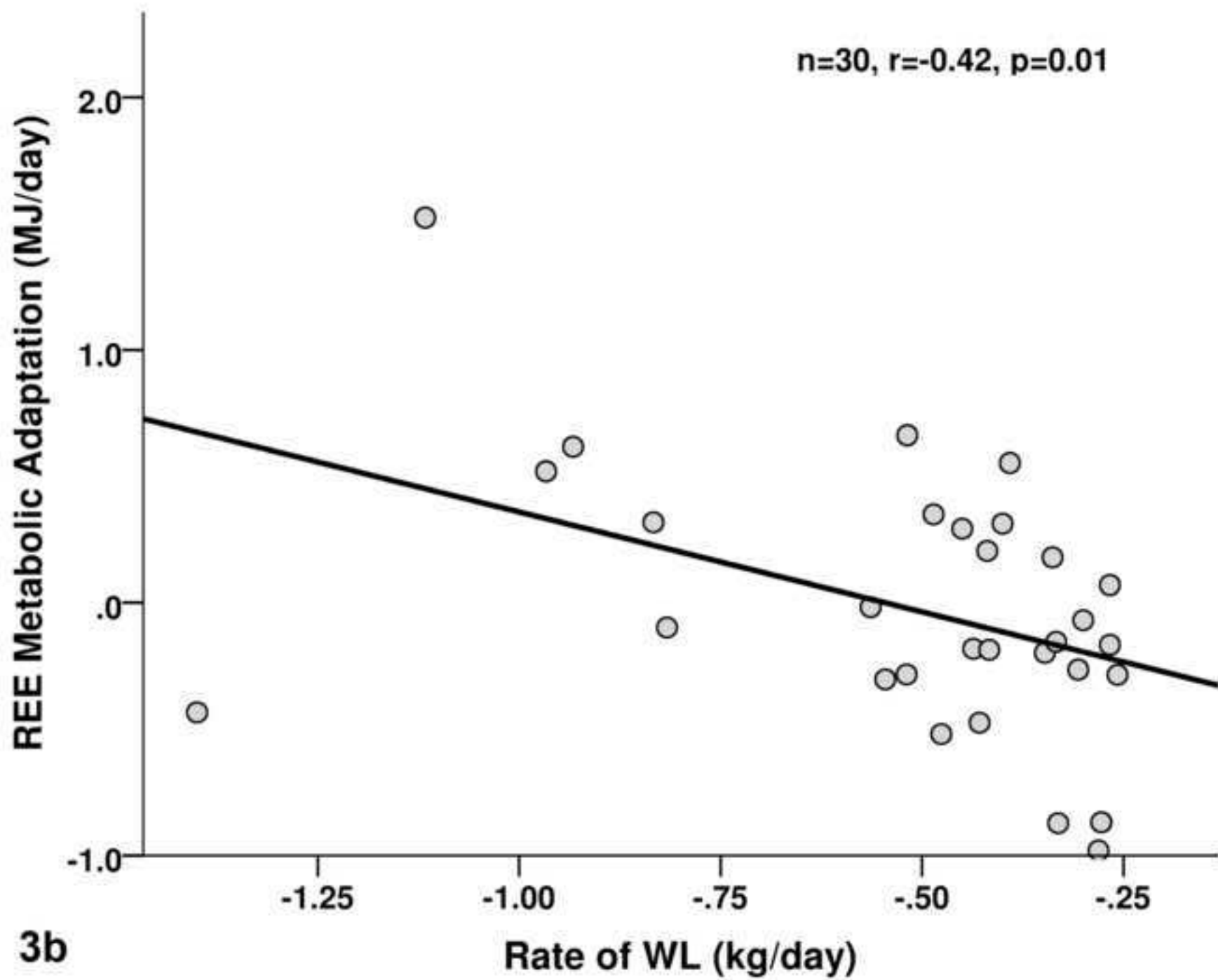


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3a



3b

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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