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Title: Associations between the proportion of fat-free mass loss during weight loss, changes in appetite and subsequent weight change: results from a randomised 2-stage dietary intervention trial

Running head: Changes in body composition and energy balance

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Abbreviations: CID – clinical investigation day; DEXA - dual-energy x-ray absorptiometry; EE – energy expenditure; EI – energy intake; FFM – fat-free mass; FFML – fat-free mass loss; FM – fat mass; LCD – low calorie diet; VAS – visual analogue scale; VLCD – very low calorie diet

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1 ABSTRACT

2 **Background:** Dynamic changes in body composition which occur during weight loss may
3 have an influential role on subsequent energy balance behaviours and weight.

4 **Objective:** The aim of this paper is to consider the effect of proportionate changes in body
5 composition during weight loss on subsequent changes in appetite and weight outcomes at 26
6 weeks in individuals engaged in a weight loss maintenance intervention.

7 **Design:** A sub-group of the Diet Obesity and Genes (DiOGenes) study (n=209) were
8 recruited from three European countries. Participants underwent an 8-week low-calorie diet
9 (LCD) resulting in $\geq 8\%$ body weight loss, during which changes in body composition (by
10 dual-energy x-ray absorptiometry) and appetite (by visual analogue scale appetite perceptions
11 in response to a fixed test meal) were measured. Participants were randomised into 5 weight
12 loss maintenance diets based on protein and glycaemic index content and followed up for 26
13 weeks. We investigated associations between the proportionate loss of fat free mass
14 (%FFML) during weight loss and (1) weight outcomes at 26 weeks and (2) changes in
15 appetite perceptions.

16 **Results:** During the LCD, participants lost a mean (SD) of 11.2 (3.5) kg of which 30.4% was
17 fat-free mass. Following adjustment, there was a tendency for %FFML to predict weight
18 regain in the whole group ($\beta=0.041$ (-0.001, 0.08), $p=0.055$) which was significant in males
19 ($\beta=0.09$ (0.02, 0.15), $p=0.009$) but not females ($\beta=0.01$ (-0.04, 0.07), $p=0.69$). Associations
20 between %FFML and change in appetite perceptions during weight loss were inconsistent.
21 The strongest observations were in males for hunger ($r=0.69$, $p=0.002$) and desire to eat
22 ($r=0.61$, $p=0.009$), with some tendencies in the whole group and no associations in females.

23 **Conclusions:** Our results suggest that composition of weight loss may have functional

24 importance for energy balance regulation, with greater losses of fat-free mass (FFM)

25 potentially being associated with increased weight regain and appetite.

26 **Key words:** Fat-free mass, fat mass, body composition, appetite, weight loss, weight regain,

27 obesity, low-calorie diet, DiOGenes

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51 **1. Introduction**

52 While weight regain following weight loss is common, there is still a great deal to
53 understand regarding factors that facilitate the maintenance of weight loss or drive weight
54 regain. Weight loss is known to influence components of energy intake (EI) and energy
55 expenditure (EE) in a manner which promotes weight regain (1). One frequently reported
56 consequence of weight loss is an increase in appetite (2–4) (with some exceptions; (5)), which
57 may lead to greater EI and weight regain. The potential physiological mechanisms by which
58 weight loss affects appetite are not fully understood, but may include changes in appetite-
59 related peptides, oro-sensory sensations, reward sensitivity and neuroendocrine systems (2).
60 Weight loss is comprised of reductions in both fat mass (FM) and fat-free mass (FFM)
61 compartments both of which may exert different effects on appetite and EI.

62 The adipostatic theory of energy balance regulation propose that leptin is a key
63 hormone mediating changes in appetite and energy intake (6) through its action on hunger and
64 satiety related hypothalamic neurons (7). In addition, studies have shown a strong and robust
65 positive association between absolute FFM and EI based on cross-sectional data in
66 individuals at approximate energy balance (8–14) with comparatively less variance in EI
67 explained by FM. The assumption therefore is that a ‘tonic’ signal exists linking FFM and EI
68 (15,16) potentially mediated by the energetic requirements of FFM (9).

69 During energy deficits there is some evidence that greater loss of FFM is associated
70 with a greater elevation of EI. Dulloo et al’s (1997) re-analysis of the Minnesota Starvation
71 Experiment suggested that the prior depletion of both FM and of FFM were interdependently
72 associated a subsequent hyperphagic response indicating that dynamic changes in the
73 proportion of FM:FFM of the body during extreme energy deficits may impact subsequent EI

74 (17) . The positive association between FFM and EI at approximate energy balance, and the
75 association between greater proportionate loss of FFM and EI subsequent to severe energy
76 deficits have been termed as passive and active influences on EI respectively (15,16). The
77 passive role may be mediated by energetic demands of the body, whereas the active role is
78 hypothesised to cause a more pronounced “drive” to increase EI in response to loss of FFM
79 during energy deficits, in a manner driven by reductions in organ and skeletal muscle mass
80 (i.e. body protein) (16).

81 To date, evidence that proportionate FFM changes are associated with hyperphagia
82 subsequent to weight loss comes from small, select studies during relatively extreme energy
83 deficits (18,19). It is unclear if such effects are apparent in those with overweight or obesity in
84 voluntary weight loss programmes. One recent study reported that greater fractional loss of
85 FFM (%FFML) was predictive of subsequent weight regain, although the mechanisms of
86 action were not investigated (20). A previous meta-analysis in 2 379 individuals with
87 overweight and obesity who lost and regained weight suggested that reductions in FM and
88 FFM during weight loss better predict subsequent weight change than weight loss alone (21).
89 The aims of this exploratory, post-hoc analysis were to 1) test the relationships of changes in
90 body composition experienced during an 8-week LCD in individuals losing $\geq 8\%$ body weight
91 on follow-up weight at 26 weeks, and 2) to test the effect of these body composition changes
92 on appetite perceptions. We hypothesised that greater proportionate reduction in FFM (in
93 comparison to FM) would result in (1) greater weight regain at follow-up and (2) greater
94 increases in appetite perceptions.

95

96 **2. Methods**

97 *2.1 Study design*

98 The present study was a post-hoc analysis of the data collected as part of the Diet, Obesity

99 and Genes (DiOGenes) study (<http://www.diogenes-eu.org/>) (22). The DiOGenes study was a
100 European multi-centre randomised control trial designed primarily to test the effect of five
101 diets (low-protein, low-glycemic index (LPLGI), high-protein, low-glycemic index (HPLGI),
102 low-protein high-glycemic index (LPHGI), high-protein, high-glycemic index (HPHGI) and a
103 control group (CON)) on weight loss maintenance outcomes over the 26-week weight loss
104 intervention following at least 8% reduction in body weight over an 8-week period achieved
105 by low calorie diet (LCD) (Modifast; Nutrition et Sante, Revel, France). The DiOGenes study
106 protocol and primary results have previously been published elsewhere (22,23). The present
107 sub-study is concerned with data collected at clinical investigation day (CID) 1 which
108 occurred prior to weight loss intervention; CID2 at the end of 8 weeks immediately following
109 the LCD and weight change at CID3, after 26 weeks of the weight loss maintenance
110 intervention. The primary aim of this post-hoc analysis was to test the effect of proportionate
111 changes in body composition incurred during an 8-week LCD on follow-up weight at 26
112 weeks and, secondarily, to test the effect of these body composition changes on weight
113 change in appetite perceptions.

114

115 *2.2 Participants*

116 All participants were recruited to the DiOGenes study between November 2005 and April
117 2007 from eight European centres, of which three had the necessary data available for the
118 present analysis and were located in Copenhagen, Denmark; Cambridge, UK; Potsdam,
119 Germany. Participants had either overweight or obesity (body mass index (BMI) between 27
120 and 45 kg/m²) and were between 18 and 65 years old. Further information on inclusion and
121 exclusion criteria can be found elsewhere (22). In the present study, to test hypothesis (1),
122 only participants who completed the 8-week LCD with at least 8% weight loss (set originally
123 by the DiOGenes study) and had dual-energy x-ray absorptiometry (DEXA) measurements at

124 CID1 and CID2 and a measurement of body weight at CID3 were included. To test
125 hypothesis (2), those eligible for (1) and had additional measurements of appetitive ratings
126 during a standardised test-meal at CID1 and CID2 were included. A participant flow diagram
127 is given in **figure 1**. Procedures followed in the DiOGenes study were in accordance with the
128 Declaration of Helsinki and approved by local ethics committees in all participating countries.
129 Written informed consent was obtained from all participants.

130

131 [insert figure 1]

132

133 *2.3 Anthropometric measures*

134 Body weight, waist circumference and body composition were measured as described
135 previously (22). Body composition was measured using a 2-compartment model (i.e. FM and
136 FFM) by DEXA.

137

138 [insert table 1]

139

140 *2.4 Standardised test meal and appetite ratings*

141 Full details of the test-meal protocol are provided elsewhere (5). Briefly, a homogenous
142 pasta-based test meal providing 1.6 MJ of energy, of which 61% of carbohydrate, 26% was
143 fat and 13% was protein was given around lunch time at CID1 and CID2. Participants were
144 requested to fast overnight before each test meal and could drink a maximum of 1 dl water
145 before the test. Participants were instructed to consume all of the test meal and were free to
146 drink water ad libitum. Visual analogue scale (VAS) ratings were obtained at 15 minutes
147 before and then at 15, 30, 60, 90, 120, 150, and 180 min after the start of the test meal. The
148 VAS for appetite measurement consisted of a series of 100 mm horizontal lines anchored with

149 extreme appetite perceptions on both ends of each line (e.g. not at all hungry-very hungry).
150 They were used to answer each of the following 4 questions: how hungry are you? (not at all
151 hungry-very hungry), how full do you feel? (not at all full-very full), how strong is your
152 desire to eat? (not at all strong-very strong), how much food do you think you can eat? (none
153 at all-a large amount) (24).

154

155 *2.5 Statistical analyses*

156 Mean and standard deviations (SD) for participant characteristics and key variables are
157 provided in **table 1**. All variables reported were assessed for normality by visual inspection of
158 QQ plots and histograms. Analyses were run for all participants and separately for each sex
159 due to known differences in body composition dynamics between sexes. Proportionate change
160 in body composition was represented by the term %FFML which represents an integrated
161 change in both compartments and not simply a change in FFM (i.e. proportionate fat mass
162 loss (%FML) = 100 - %FFML). Percentage FFML was calculated as the change in FFM
163 during weight loss divided by total weight loss (i.e. $(\Delta\text{FFM}/\Delta\text{weight}) * 100$) (20,25).
164 Percentage FFML values above 80% (n=5) were removed due to this being the greatest
165 reported %FFML which was observed under conditions of semi-starvation in lean individuals
166 (26), therefore were considered erroneous measures. Absolute weight loss was chosen over
167 relative weight loss as we were investigating relative body composition and therefore using
168 absolute weight improves interpretability of body composition changes. Student's t-tests and
169 chi-squared tests were conducted to test differences between sexes for continuous and
170 categorical variables respectively. We also examined the associations between baseline body
171 fat and %FFML for both sexes in line with previous observations (26,27) which can be found
172 in **supplementary figure 1**.

173 Next, Pearson correlations were conducted between predictor, outcome and covariate

174 variables related to initial and changes in body composition and weight. Pearson correlation
175 was chosen based on visual inspection of the distribution of the variables through histogram
176 and Q-Q plots which were deemed to be parametric (28). Next, univariate linear regressions
177 were conducted to investigate crude associations between predictor and the outcome
178 variables. Beta coefficients were reported as unstandardized estimates and 95% confidence
179 intervals, representing the estimate and confidence of 1-unit change in predictor variable per
180 1kg change in weight outcomes at 26 weeks. Next, multivariate linear regression models were
181 generated for all individuals and by gender. The models were adjusted for dietary arm and
182 trial centre due to previously observed effects on weight loss maintenance (22,29). Further
183 adjustments were made for the amount of weight lost (as strong associations between weight
184 lost and regained has been shown previously; (21)), as well as initial body weight and body
185 fat, as proportionate changes in body composition are known to be influenced by initial body
186 size (26,27) and FFM loss may be more pronounced in leaner individuals, with potential
187 effects exerted on energy balance regulation (17). Lastly, we probed for interaction effects
188 between sex and the primary predictor (%FFML) in these multivariate models. Collinearity
189 and multicollinearity were tested by examining the variance inflation factors (VIF) of the
190 model variables which are reported in **supplementary table 6** (of which none were deemed to
191 be highly covaried). Scatterplots were produced to visualise main effects.

192 To test whether differences in the composition of weight loss were associated with
193 changes in appetite measured over the duration of a standardized test meal, we calculated the
194 total area under the curve (AUC) using the trapezoid method (31) consistent with a previous
195 analysis of this data (5). Change in total AUC for hunger, fullness, desire to eat and
196 prospective consumption between CID1 and CID2 (i.e. CID2 – CID1) were calculated.
197 Lastly, the association between %FFML and change in appetite perceptions was assessed
198 using Pearson correlation following visual inspection of QQ plots and histograms by which

199 they were deemed parametric. In a final step, we considered the effect of appetitive changes
200 in the available group of weight change at 26 weeks by Pearson correlation. This sub-analysis
201 is documented in **supplementary analysis 1** with results provided in **supplementary table 4**.
202 In addition to examining proportionate change in body composition, we also considered
203 independent relative change in both FM and FFM compartments from baseline and used these
204 variables to predict both (1) weight regain at 26 weeks and (2) changes in appetite
205 perceptions. The specifics of this analysis are expanded in **supplementary analysis 2** and
206 results are provided in **supplementary table 5** and **supplementary figure 2-3**. Since the
207 present study is a post-hoc exploratory analysis, adjustment for the testing of multiple
208 outcomes was not employed (30). All significance testing, unless otherwise stated, was
209 performed using an alpha level of 0.05. All statistical analyses were conducted in R version
210 3.5.1 (www.r-project.org).

211

212 3. Results

213 3.1 Participant Characteristics

214 Baseline characteristics are reported in **table 1**. A total of 209 participants were included
215 in the primary analysis, of which 132 were females. There was no difference in age between
216 sexes. Males were heavier and had greater FFM than females at baseline, and females had
217 greater FM than males. Males lost greater amounts of absolute (13.0 (4.0) vs 10.1 (2.7) kg,
218 $p<0.001$) and relative (12 (3.3) vs 10.7 (2.4) %, $p=0.002$) weight than females, of which a
219 greater proportion was FFM (35.3 (16.3) vs 27.5 (15.8) %, $p<0.001$). Lastly, males regained
220 more weight during the 26-week follow up period than females (2.9 (4.7) vs 0.8 (4.7) kg,
221 $p=0.001$).

222

223 [insert table 1]

224 3.2 Weight change at 26 weeks

225 Pearson correlations between predictor, covariates and weight change at 26-week follow-
 226 up are provided in **supplementary tables 1-3** for all participants, males and females
 227 respectively. Univariate regression results predicting weight change at 26 weeks are provided
 228 in **table 2**. In the total group, the amount of weight lost ($\beta=0.267$ (0.086, 0.448) kg, $R^2=3.3\%$)
 229 significantly predicted weight change, and the fraction of weight lost as FFM tended towards
 230 a significant association ($\beta=0.038$ (-0.001, 0.078) %, $R^2=1.8\%$). In males, the amount of
 231 weight ($\beta=0.401$ (0.148, 0.654) kg, $R^2=11.4\%$) and %FFM ($\beta=0.070$ (0.006, 0.134) %, $R^2=4.6\%$)
 232 lost during weight loss were significantly associated with subsequent weight
 233 change. In females, weight loss ($\beta=0.544$ (0.264, 0.824) kg, $R^2=10.0\%$) but not %FFM
 234 ($\beta=0.002$ (-0.049, 0.052) %, $R^2=0.1\%$) lost was associated with subsequent weight regain.
 235 The relationship between %FFML and subsequent weight change at 26 weeks is shown in
 236 **figure 2**.

237

238 [Insert table 2]

239 [Insert figure 2]

240 Results from a multivariate linear model are reported in **table 3**. Following adjustment,
 241 weight loss ($\beta=0.57$ (0.36, 0.77) kg) was associated with weight change at follow-up and
 242 %FFML showed a tendency towards significance ($\beta=0.041$ (-0.001, 0.08) %, $R^2=1.8\%$). In
 243 males, both weight loss ($\beta=0.65$ (0.40, 0.90) kg) and %FFML ($\beta=0.09$ (0.02, 0.15) %, $R^2=6.5\%$)
 244 were positively associated with weight change. In females, weight loss ($\beta=0.56$
 245 (0.20, 0.92) kg) but not %FFML affected subsequent weight outcomes ($\beta=0.01$ (-0.04, 0.07)
 246 %, $R^2=0.1\%$). As a final step, we entered the interaction term (sex \times %FFML) into the full
 247 model, which showed a tendency towards a sex interaction ($p=0.056$).

248

249 3.3 Changes in appetite

250 Changes in subjective appetite in response to a standardised test meal before and after
251 weight loss are reported in **table 4** which were indicative of an overall decrease in appetite.
252 Associations between changes in appetite and %FFML during weight loss are illustrated in
253 **figure 3**. In the total group, there was evidence of a weak positive association with %FFML
254 and change in hunger ($r=0.28$, $p=0.07$) and a weak negative association with change in
255 fullness ($r=-0.30$, $p=0.054$). Associations between %FFML and desire to eat ($r=0.20$, $p=0.20$)
256 or prospective consumption ($r=0.09$, $p=0.71$) were non-significant. In males, there was a
257 significant positive association between %FFML and change in hunger ($r=0.69$, $p=0.002$) and
258 desire to eat ($r=0.61$, $p=0.009$) and a weaker association with change in prospective
259 consumption ($r=0.34$, $p=0.17$). Lastly in males, a strong negative association with change in
260 fullness ($r=-0.55$, $p=0.02$) was observed. In females, non-significant associations between
261 %FFML and change in hunger ($r=0.25$, $p=0.24$) and fullness ($r=-0.25$, $p=0.26$) were observed.
262 No significant associations between %FFML and change in desire to eat ($r=0.18$, $p=0.39$) or
263 prospective consumption ($r=0.02$, $p=0.94$) were observed in females.

264

265 [insert figure 3]

266 4. Discussion

267 This study observed a positive association between %FFML and subsequent weight
268 change at 26 weeks, which was significant in males but not females. Positive associations
269 between %FFML and changes appetite collected during a test meal before and after weight
270 loss were inconsistent overall though stronger in males.

271 Body composition has long been associated with energy balance regulation, although
272 primarily as a key determinant of EE that explains up to 93% of the variance in RMR when
273 the size and composition of functional body compartments are determined (32). The

274 interaction between dynamic changes in body composition during weight loss and regain has
275 not been well studied. While evidence suggests FFM and EI are positively associated in
276 individuals in approximate energy balance, weight loss decreases FFM but usually increases
277 appetite (4,33).

278 To explain this paradox, Dulloo et al (2017) proposed two discrete homeostatic
279 control systems that operate through FFM and act on EI: a passive or 'tonic' positive
280 relationship which provides homeostatic matching of EI and EE via energy requirements; and
281 an active drive, triggered by reductions in the size and structural integrity of FFM
282 (specifically, protein from muscle and organs) which increases EI (16) (17). Evidence to
283 support this contention is sparse, stemming from the Minnesota starvation study (18), and
284 more recently from Vink et al. who reported an association between %FFML after 8-9kg
285 weight loss, and subsequent weight regain after 9 months (20). Also, a recent meta-regression
286 reported that integrated changes in FFM and FM explained greater variance in subsequent
287 weight response than weight loss alone (29% vs 40%, respectively) (21) suggesting functional
288 roles of both compartments above that of weight alone.

289 In the present study, we found evidence of longitudinal associations between %FFML
290 and subsequent weight change in males but not females. Sex differences may be because
291 %FFML was lower in females than in males (35% vs 27% respectively), potentially due to
292 the significant negative association which between %FFML and initial body fat
293 (supplementary figure 1) (27). The hypothetical 'active drive' in EI (and hence weight regain)
294 generated by reductions in FFM would be more pronounced in leaner individuals and males
295 due to higher %FFML with weight loss (34). After adjusting for total weight loss, initial
296 weight and body fat, addition of %FFML explained an additional 6.4% ($p=0.009$) of the
297 variance in weight regain in men and 1.2% ($p=0.055$) in the total group, but no effect was
298 seen in females. Weight loss was positively associated with weight regain in all analyses,

299 however, the composition of this loss (specifically, %FFML) also seemed to explain
300 additional variance, suggesting a functional role of FFM in energy balance regulation.
301 Another interpretation of the present results is that greater %FML is associated with less
302 weight regain which is inconsistent with models suggesting that reduction in adipose tissue is
303 the primary determinant of weight regain via neuroendocrine adaptations (35). However,
304 when we examined the independent change in FFM and FM compartments from baseline
305 values during the LCD (see supplementary analysis 2), change in FM from baseline was not
306 associated with weight or appetitive outcomes, though change in FFM from baseline showed
307 a tendency towards weight and appetite effects in some cases (again only in male
308 participants).

309 The mechanism by which %FFML may be predictive of weight regain is unclear.
310 Greater loss of protein tissues (e.g. muscle and organs) causes greater reductions in EE, but
311 the effect of these changes on appetite and EI are less understood. Increased appetite
312 following weight loss is observed in some (4,36,37), but not all studies (5,38). Indeed, in a
313 previous analysis of the test meal VAS data used in the DiOGenes trial, post-prandial appetite
314 in response to the test meal was decreased in response to weight loss (5). Possible
315 explanations included: (i) participants were still in a negative energy balance at the time of the
316 second measure; (ii) reductions in gastrointestinal transit time following the 8-week LCD;
317 (iii) psychological habituation to smaller portion sizes, resulting in greater satiety from the
318 test-meal following the LCD.

319 Similar to Andreissen et al., we observed an overall decrease in appetite during weight
320 loss in our sub-sample. Sex differences in the relationship between %FFML and appetite were
321 evident, with stronger correlations in males and weak-to-no correlations in females,
322 potentially driven by the greater %FFML observed in male participants or the smaller overall
323 change in appetite between visits in females. To our knowledge only one study has considered

324 the effect of FFML on change in EI during extreme weight loss in lean individuals (17). The
325 current study suggests that the effect of %FFML on subsequent appetite is evident (albeit
326 relatively weak) in individuals with overweight and obesity undergoing therapeutic weight
327 loss. It has been suggested that signals released from protein tissue such as organ and skeletal
328 muscle (referred to as proteinstats) during weight and FFM loss may act on higher centres to
329 produce this effect (34). In a further step, we examined whether change in appetite predicted
330 weight change at 26 weeks (supplementary analysis 1). All correlations were generally in a
331 direction representative of increased weight change in response to greater increases in
332 appetite, however, most were non-significant, potentially due to the small sample sizes
333 available.

334 The fraction of weight lost as FFM is known to be affected by the magnitude of the
335 energy deficit, with diets such as VLCDs producing proportionately larger reductions in FFM
336 than less severe caloric restrictions (25). The mean %FFML in the present study (30.4%) was
337 comparable to that reported by several other studies (31-37%) in which weight reduction was
338 achieved by LCD or VLCD in similar populations with similar weight losses (39–42). In the
339 present study we observed large deviations from the mean, with many individuals losing over
340 50% of their weight as FFM, and some gaining FFM during weight loss (a phenomenon also
341 observed by Vink et al. (2016)). We removed individuals with %FFML > 80% (n=5) due to
342 this being the greatest recorded %FFML [26], assuming measurement error or substantial
343 water flux might explain these observations. Removing these outliers decreased apparent
344 effects of the relationships between %FFML and weight regain reported here. Furthermore,
345 there was a negative association between %FFML and weight loss. This can be explained by
346 the rapid losses of FFM in the initial phase of weight loss which slows over time, as described
347 previously (43,44). However, as we adjusted for both changes in body composition and total
348 weight loss this association does not confound the observed effect.

349 It is hypothesised that the functional effect of FFM on EI is activated by a threat to the
350 structure of organ and skeletal muscle tissue (i.e. protein) by prolonged negative energy
351 balance. Early work by Dulloo was based on FFM measurements which were adjusted for
352 hydration and relative bone mass (45). However, 2-compartment models of body composition
353 fail to differentiate between protein, water, mineral, carbohydrate and other components of
354 FFM. In the first few weeks of weight loss, changes in total body water are likely to
355 contribute disproportionately to FFML (44). Therefore, it is difficult to relate changes in 2-
356 compartment models to the functional properties of specific components of these
357 compartments. To further understand the functional role of body composition in various states
358 of energy balance, higher resolution body composition models should be used to estimate the
359 fractional contribution of water and protein to FFML and estimate change in individual organ
360 weights and mineral mass (46). Such models, longitudinally aligned with repeated
361 measurements of appetite, EI or EE may allow cause-effect relationships between changes in
362 body structure and components of energy balance that resist weight loss or promote weight
363 regain to be determined.

364 We were limited to individuals with overweight and obesity. It is likely that a similar
365 percentage of weight loss in a sample with healthy weight would have led to a greater
366 %FFML, which may have had a more pronounced impact on appetite and weight regain.
367 Further, we used repeated VAS scores during a fixed test meal to assess appetite, however,
368 further research using *ad libitum* EI may provide greater mechanistic insight. We were limited
369 in sample size for the analysis relating to appetite which was therefore constrained to basic
370 correlative associations and meant we were unable to examine whether changes in appetite
371 perceptions mediated the relationship between %FFML and weight change. Models may have
372 been better informed by inclusion of physical activity measurements during the weight loss
373 and maintenance phases due to interactions between activity, weight and body composition,

374 though no accurate and consistent measure was available. Moreover, information on loss to
375 follow-up and withdrawal were unavailable in this specific sub-sample and may have affected
376 observed outcomes. A range of unmeasured physiological, cognitive and behavioural factors
377 may also have affected the observed outcomes though we were unable to adjust for these in
378 our models. Lastly, a 2-compartment model to assess longitudinal changes in body
379 composition, used change in FFM as a proxy for change in skeletal muscle and organ weights
380 which are likely to be the functional component of FFM.

381 These data suggest that the composition of weight loss may contribute to
382 physiological resistance to weight loss via appetitive responses, but under the conditions of
383 this study the effect was small and variable. In the whole population and males and females
384 separately, the amount of weight loss was a predictor of weight regain. Functional effects of
385 %FFML on subsequent appetite and weight regain were evident in males, but not in females.
386 These data are consistent with Dulloo's model of an active drive from %FFML elevating
387 appetite (34). Relationships between changes in functional body composition, (measured
388 using more advanced methods and models) and energy balance behaviours warrant further
389 investigation.

390

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412

413 **Data described in the manuscript, code book, and analytic code will be made available**
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418 **Conflicts of interest**

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Tables

Table 1. Subject Characteristics

	Total (n=209)	Male (n=77)	Female (n=132)	p-value
	209	77	132	
Age (years)	42.4 (5.7)	42.3 (5.8)	42.5 (5.6)	0.804
Country (%)				0.283
Denmark	95 (45.5)	38 (49.4)	57 (43.2)	
UK	51 (24.2)	13 (16.9)	38 (28.8)	
Germany	63 (30.1)	26 (33.8)	37 (28.0)	
Diet Arm (%)				0.544
LPLGI	39 (18.7)	13 (16.9)	26 (19.7)	
LPHGI	34 (16.3)	10 (13.0)	24 (18.2)	
HPLGI	51 (24.4)	23 (29.9)	28 (21.2)	
HPHGI	42 (20.1)	17 (22.1)	25 (18.9)	
CTR	43 (20.6)	14 (18.2)	29 (22.0)	
Baseline weight (kg)	99.58 (16.25)	108.78 (14.84)	94.21 (14.58)	<0.001
Baseline FM (kg)	40.07 (10.25)	36.12 (9.49)	42.37 (10.00)	<0.001
Baseline FFM (kg)	59.05 (12.40)	72.26 (8.03)	51.34 (6.64)	<0.001
Baseline bodyfat (%)	40.26 (7.60)	32.84 (5.18)	44.59 (4.99)	<0.001
Baseline FFM (%)	59.74 (9.21)	67.16 (4.12)	55.41 (3.11)	<0.001
Weight loss (kg)	-11.17 (3.52)	-13.04 (3.96)	-10.08 (2.71)	<0.001
Weight loss (%)	-11.19 (2.84)	-11.99 (3.30)	-10.72 (2.43)	0.002
FFML (%)	30.37 (16.38)	35.31 (16.29)	27.49 (15.79)	<0.001
Weight change at 26 weeks (kg)	1.57 (4.78)	2.94 (4.71)	0.77 (4.65)	0.001

Table 1 legend: Baseline characteristics collected at clinical investigation day 1. Mean (SD) are reported for absolute values or percentages where stated. Weight loss was calculated as the difference before and after the dietary intervention, and relative weight loss was this value

as a percentage of baseline weight. Percentage fat-free mass loss (%FFML) was calculated as the fraction of weight lost as FFM (i.e. $(\Delta\text{FFM}/\Delta\text{weight}) * 100$) during the dietary intervention.

Abbreviations: control (CTR); fat-free mass (FFM), fat mass (FM); high-protein, low glycemic index (HPLGI), high-protein, high-glycemic index (HPHGI); low-protein, low-glycemic index (LPLGI), low-protein high-glycemic index (LPHGI); percentage fat-free mass loss (%FFML). P-values denote results of students t-tests for continuous variables and chi-squared tests for categorical variables between males and females.

Table 2. Univariate regression analyses predicting weight regain at 26 weeks

Predictor	All (n=209)			Males (n=77)			Females (n=132)		
	β Coefficient (95% CI)	P-value	R ²	β Coefficient (95% CI)	P-value	R ²	β Coefficient (95% CI)	P-value	R ²
Age (years)	0.091 (-0.023, 0.204)	0.119	1.2%	0.043 (-0.14, 0.226)	0.747	0.3%	0.126 (-0.014, 0.266)	0.081	2.3%
Baseline weight (kg)	0.020 (-0.020, 0.060)	0.326	0.5%	0.031 (-0.041, 0.102)	0.263	1.6%	-0.034 (-0.088, 0.021)	0.224	1.1%
Baseline FFM (kg)	0.085 (0.034, 0.136)	0.002	4.8%	0.107 (-0.024, 0.237)	0.080	3.3%	-0.003 (-0.124, 0.117)	0.959	0%
Baseline FM (kg)	-0.075 (-0.138, -0.012)	0.020	2.6%	-0.009 (-0.122, 0.103)	0.968	0%	-0.070 (-0.149, 0.009)	0.082	2.2%
Baseline body fat (%)	-0.160 (-0.243, -0.077)	<0.001	6.5%	-0.133 (-0.337, 0.071)	0.205	2.1%	-0.126 (-0.285, 0.033)	0.122	1.8%
Weight loss during LCD (kg)	0.267 (0.086, 0.448)	<0.001	3.3%	0.401 (0.148, 0.654)	<0.001	11.4%	0.544 (0.264, 0.824)	<0.001	10.0%
FFML (%)	0.038 (-0.001, 0.078)	0.059	1.8%	0.070 (0.006, 0.134)	0.039	4.6%	0.002 (-0.049, 0.052)	0.949	0.1%

Table 2 legend. Univariate linear regression analyses predicting weight change at 26 months in 209 individuals following weight loss. Each unstandardised beta-coefficient represents 1kg weight change at 26 weeks per unit of the predictor. For example, a beta value of 0.27 (0.09, 0.45) kg for weight loss means that for every 1kg of weight regained, an average of 0.27 kg (ranging from 0.09 – 0.45kg of weight was lost). For categorical variables these represent difference from the reference group. Weight loss was calculated as the difference before and after the dietary intervention, and relative weight loss was this value as a percentage of baseline weight. Percentage fat-free mass loss (%FFML) was calculated as the fraction of weight lost as FFM (i.e. $(\Delta\text{FFM}/\Delta\text{weight}) \times 100$) during the dietary intervention. Abbreviations; fat-free mass (FFM), fat mass (FM), fat-free mass loss (FFML), LCD (low-calorie diet).

Table 3. Multivariate linear regression models predicting weight regain at 26 weeks

	All (n=209)			Males (n=77)			Females (n=132)		
	β Coefficient (95% CI)	P-value	Adjusted R ² ¹	β Coefficient (95% CI)	P-value	Adjusted R ² ¹	β Coefficient (95% CI)	P-value	Adjusted R ² ¹
Multivariate model ²		<0.001	20.8%		<0.001	32.5%		0.007	11.2%
Trial centre									
Denmark ³	0			0			0		
UK	2.53 (0.98, 4.08)	0.002		4.25 (1.64, 6.87)	0.002		1.60 (-0.40, 3.61)	0.120	
Germany	-0.02 (-1.51, 1.47)	0.981		-0.89 (-3.15, 1.36)	0.44		0.78 (-1.45, 3.01)	0.493	
Dietary arm									
Control ³	0			0			0		
LPLGI	-0.61 (-2.46, 1.24)	0.519		-2.45 (-5.58, 0.69)	0.131		0.07 (-2.31, 2.45)	0.951	
LPHGI	-0.28 (-2.22, 1.67)	0.780		-1.69 (-4.95, 1.57)	0.312		0.20 (-2.25, 2.65)	0.873	
HPLGI	0.29 (-1.43, 2.00)	0.746		-0.53 (-3.14, 2.09)	0.695		0.71 (-1.61, 3.04)	0.548	
HPHGI	0.92 (-0.91, 2.74)	0.325		-0.89 (-3.70, 1.92)	0.537		1.79 (-0.64, 4.22)	0.151	
Baseline weight (kg)	0.08 (0.04, 0.13)	<0.001		0.14 (0.06, 0.23)	0.001		0.04 (-0.04, 0.11)	0.302	

Baseline body fat (%)	-0.20 (-0.28, -0.11)	<0.001		-0.37 (-0.61, -0.14)	0.002		-0.05 (-0.27, 0.16)	0.653	
Weight loss (kg)	0.57 (0.36, 0.77)	<0.001		0.65 (0.40, 0.90)	<0.001		0.56 (0.20, 0.92)	0.003	
%FFML	0.041 (-0.001, 0.08)	0.055	1.8%	0.09 (0.02, 0.15)	0.009	6.5%	0.01 (-0.04, 0.07)	0.690	0.1%

Table 3 legend. Multivariate linear regression model predicting weight change at 26 weeks. ¹R² for %FFML denotes the change in variance explained when %FFML is added to the model. ²Model adjusted for dietary arm and trial centre. ³reference group for a given categorical variable. Each unstandardised beta-coefficient represents 1kg weight change at 26 weeks per unit of the predictor variable. For example, a beta value of 0.57 (0.36, 0.77) kg for weight loss means that for every 1kg of weight regained, an average of 0.57 kg (ranging from 0.36 – 0.77kg of weight was lost). For categorical variables these represent difference from the reference group. Abbreviations; Control (CTR); fat-free mass loss (FFML); high-protein, low glycemic index (HPLGI), high-protein, high-glycemic index (HPHGI); low-protein, low-glycemic index (LPLGI), low-protein high-glycemic index (LPHGI)

Table 4. Self-reported appetite perceptions measured by visual analogue scale in response to a fixed test meal

	All (n=40)				Males (n=17)				Females (n=23)			
	CID1	CID2	Change	p-value	CID1	CID2	Change	p-value	CID1	CID2	Change	p-value
Hunger	6144 (3884)	4626 (3004)	-1518 (3068)	0.051	7926 (3737)	6102 (2890)	-1825 (2644)	0.123	4876 (3615)	3670 (2695)	-1207 (3417)	0.231
Fullness	10876 (4106)	12497 (3115)	1620 (3695)	0.048	9719 (3950)	11320 (3008)	1601 (3653)	0.191	11651 (4182)	13217 (2986)	1566 (3874)	0.150
Desire	6725 (3947)	4837 (2818)	-1888 (3381)	0.015	8435 (3671)	5940 (2762)	-2495 (2864)	0.034	5531 (3829)	4126 (2677)	-1405 (3776)	0.151
Prospective	7071 (3718)	4929 (2853)	-2144 (3351)	0.004	9044 (3286)	6407 (2488)	-2637 (2878)	0.014	5640 (3481)	3970 (2681)	-1671 (3699)	0.070

Table 4 legend. Visual analogue scale ratings for hunger, fullness, desire to eat and prospective consumption given as mean (SD) calculated as the total area under the curve by trapezoid method summing 8 repeated measures beginning 15 minutes before and ending 180 minutes after a fixed pasta-based test meal. P-values denote significant differences between clinical investigation days 1 and 2 in each group as tested by students t-test. Abbreviations; clinical investigation day 1 (CID1) (before dietary intervention); clinical investigation date 2 (CID2) (after dietary intervention).

Figure 1 legend. Participant flow diagram detailing recruitment and participation in clinical investigation days. The number of participants with data available for analysis 1 (the effect of percentage fat-free mass loss (%FFML) on weight change at 26 weeks) and 2 (the effect of %FFML on change in appetite perceptions in response to a fixed test meal) are provided. Abbreviations: clinical investigation day (CID); dual-energy x-ray absorptiometry (DXA); fat-free mass loss (FFML); low-calorie diet (LCD); visual analogue scale (VAS).

Figure 2 legend. Scatterplot and unadjusted linear trendlines showing associations between the proportion of weight lost as fat-free mass (as measured by dual-energy x-ray absorptiometry (DXA) before and after an 8-week low calorie diet(LCD)) and subsequent weight change at 26 weeks follow up in all participants (dashed black line), males (black circles; solid black line) and females (grey triangles; dotted grey line). Abbreviations; percentage fat-free mass loss (%FFML).

Figure 3 legend. Scatterplots and unadjusted linear trendlines showing associations between the proportion of weight lost as fat-free mass during an 8-week low calorie diet (LCD) and changes in appetite during the 8 weeks. Results are reported for hunger (red), fullness (green), desire to eat (blue) and prospective consumption (purple). Figure (A) represented results in the total group (n=40); (B) represents the results in males (n=17) and (C) represents the results in females (n=23). Scores were calculated as the total difference in area under of curve from 8 repeated measures around a fixed test meal, and change scores were calculated as the difference between clinical investigation day 1 and 2. Abbreviations; area under curve (AUC), percentage fat-free mass loss (%FFML); visual analogue scale (VAS),