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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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ABSTRACT

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

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

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

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

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

importance for energy balance regulation, with greater losses of fat-free mass (FFM) potentially being associated with increased weight regain and appetite.

Key words: Fat-free mass, fat mass, body composition, appetite, weight loss, weight regain, obesity, low-calorie diet, DiOGenes

1. Introduction

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

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

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

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

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

2. Methods

2.1 Study design

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

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

2.2 *Participants*

All participants were recruited to the DiOGenes study between November 2005 and April 2007 from eight European centres, of which three had the necessary data available for the present analysis and were located in Copenhagen, Denmark; Cambridge, UK; Potsdam, Germany. Participants had either overweight or obesity (body mass index (BMI) between 27 and 45 kg/m^2) and were between 18 and 65 years old. Further information on inclusion and exclusion criteria can be found elsewhere (22). In the present study, to test hypothesis (1), only participants who completed the 8-week LCD with at least 8% weight loss (set originally by the DiOGenes study) and had dual-energy x-ray absorptiometry (DEXA) measurements at

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

[insert figure 1]

2.3 Anthropometric measures

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

[insert table 1]

2.4 Standardised test meal and appetite ratings

Full details of the test-meal protocol are provided elsewhere (5). Briefly, a homogenous pasta-based test meal providing 1.6 MJ of energy, of which 61% of carbohydrate, 26% was fat and 13% was protein was given around lunch time at CID1 and CID2. Participants were requested to fast overnight before each test meal and could drink a maximum of 1 dl water before the test. Participants were instructed to consume all of the test meal and were free to drink water ad libitum. Visual analogue scale (VAS) ratings were obtained at 15 minutes before and then at 15, 30, 60, 90, 120, 150, and 180 min after the start of the test meal. The 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. (ΔFFM/Δweight)*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

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

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

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

3. Results

3.1 Participant Characteristics

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

[insert table 1]

3.2 Weight change at 26 weeks

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

[Insert table 2]

[Insert figure 2]

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

3.3 Changes in appetite

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

[insert figure 3]

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Data described in the manuscript, code book, and analytic code will be made available upon request pending application to the DiOGenes trial

Conflicts of interest

Prof.Arne Astrup reports grants from European Community Contract no. FP6-2005513946, during the conduct of the study; personal fees from Global Dairy Platform, personal fees from McDonalds, USA, personal fees from McCain Foods, USA, personal fees from Lucozade Ribena Suntory, UK, personal fees from Weight Watchers, USA, personal fees from NestlÈ Research Center, Switzerland, personal fees from Gelesis, USA, personal fees from Swedish

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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. (ΔFFM/Δweight)*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. (ΔFFM/Δweight)*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. [1]R2 for %FFML denotes the change in variance explained when %FFML is added to the model. [2] Model adjusted for dietary arm and trial centre. [3] reference 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 summating 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),