The Journal of Nutrition 153 (2023) 1330–1337

THE JOURNAL OF NUTRITION

journal homepage: www.journals.elsevier.com/the-journal-of-nutrition

Obesity and Eating Disorders

Association between Fat-Free Mass Loss, Changes in Appetite, and Weight Regain in Individuals with Obesity

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A B S T R A C T

Background: The role of fat-free mass loss (FFML) in modulating weight regain in individuals with obesity, as well as the potential mechanisms involved, remain inconsistent.

Objectives: The aim of this study was to determine if % FFML following weight loss (WL) is a predictor of weight regain and to investigate the association between %FFML and changes in appetite markers.

Methods: Seventy individuals with obesity (BMI: 36 ± 4 kg/m^2; age: 44 ± 9 y; 29 males) underwent 8 wk of a very low energy diet (550–660 kcal/d), followed by 4 wk of gradual refeeding and weight stabilization and a 9-mo maintenance program (eucaloric diet). The primary outcomes were body weight and body composition (fat mass and fat-free mass). The secondary outcomes were plasma concentrations of β-hydroxybutyrate (a marker of ketosis) in fasting and appetite-related hormones (ghrelin, glucagon-like peptide 1, peptide YY, and cholecystokinin) and subjective appetite feelings during fasting and every 30 min after a fixed breakfast for 2.5 h. All were measured at baseline, week 9, and 1 y [week 13 in 35 subjects (25 males)]. The association between FFML, weight regain, and changes in appetite was assessed by linear regression.

Results: WL at week 9 was 17.5 ± 4.3kg and %FFML 20.4 ± 10.6%. Weight regain at 1 y was 1.7 ± 8.2 kg (8.8 ± 45.0%). After adjusting for WL and fat mass at baseline, %FFML at week 9 was not a significant predictor of weight regain. Similar results were seen at week 13. The greater the %FFML at week 9, but not 13, the smaller the reduction, or greater the increase in basal ghrelin concentration (β: −3.2; 95% CI: −5.0, −1.1; $P = 0.003$), even after adjusting for WL and β-hydroxybutyrate.

Conclusions: %FFML was not a significant predictor of weight regain at 1 y in individuals with obesity. However, a greater %FFML was accompanied by a greater increase in ghrelin secretion under ketogenic conditions, suggesting a link between fat-free mass and appetite regulation.

This trial was registered at clinicaltrials.gov as NCT01834859.

Keywords: fat-free mass, appetite, weight loss, weight regain, ghrelin, hunger

Introduction

Cross-sectional research accumulated over the last 2 decades has consistently shown that under energy balance (EB), fat-free mass (FFM) is positively associated with both energy intake (EI) and hunger [1,2]. Paradoxically, studies of energy deficit show that loss of FFM is also associated with greater hunger and EI [3,4]. Although putative feedback signals arising from adipose tissue, in particular, leptin, are commonly assumed to provide the feedback between the body's long-term energy needs and EI, a growing body of evidence suggests that lean body or FFM also plays a role in the drive to eat [1,5,6]. FFM is likely to modulate EI and body weight both indirectly via its effects on total [7] and resting energy expenditure (EE) [8–10] and directly through feedback signaling between FFM and brain regions involved in appetite control [11–13]. Therefore, the loss of FFM resulting from energy-restricted diets may contribute to

Abbreviations: AG, active ghrelin; βHB, β-hydroxybutyrate; CCK, cholecystokinin; EB, energy balance; EI, energy intake; FFM, fat-free mass; %FFML, % fat-free mass loss; FM, fat mass; GLP-1, glucagon-like peptide 1; PFC, prospective food consumption; PYY, peptide YY; VLED, very low energy diet; WL, weight loss.

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https://doi.org/10.1016/j.tjnut.2023.03.026

Received 7 February 2023; Received in revised form 16 March 2023; Accepted 21 March 2023; Available online 23 March 2023

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weight regain, not only due to lower EE, but also to the body's attempt to restore FFM by overeating.

Dulloo et al. [14] showed in their reanalysis of the Minnesota Starvation Experiment that the greater the initial loss of FFM, the greater the hyperphagic response during refeeding, even after adjusting for loss of fat mass (FM). Furthermore, hyperphagia persisted despite complete recovery of body weight and FM until FFM was completely restored to prestarvation levels. Similar findings were reported in studies of Army Rangers undergoing 2 mo of training and up to 12% weight loss (WL) [15,16]. This suggests that poststarvation hyperphagia is determined by autoregulatory feedback mechanisms, not only from fat but also from lean tissues. However, these studies were performed in lean men. The potential role of FFM loss during weight reduction in modulating long-term WL maintenance in individuals with obesity remains inconsistent, but the overall evidence suggests that loss of FFM increases the risk of weight regain [17–20]. We have recently shown that % FFM loss (%FFML) is a significant predictor of weight regain at 1 y follow-up in premenopausal women with overweight, independent of the initial WL intervention (diet alone, diet plus resistance training, or diet plus aerobic training) [17]. Vink et al. [18] also showed in 55 individuals with overweight or obesity that a greater %FFML, following diet-induced WL, was predictive of subsequent weight regain at 9-mo follow-up, even after adjusting for FM% at baseline. Moreover, a meta-analysis of more than 2000 individuals with overweight and obesity suggested that reductions in FM and FFM during WL better predict subsequent weight change than WL alone. Both %FM loss and %FFML were significant predictors of weight regain, even after adjusting for baseline BMI [19].

The exact mechanisms through which FFML modulates weight regain remain to be fully elucidated; however, it has been suggested that changes in the size and functional integrity of FFM may influence appetite and EI [14,21]. A recent reanalysis of the DiOGenes project by Turicchi et al. [20] showed that %FFML was able to predict weight regain at 6 mo follow-up in men, but not in women. Even though the association between %FFML and changes in appetite feelings during WL was overall inconsistent, in men a larger %FFML was associated with a larger increase in postprandial hunger and desire to eat. The pathways through which %FFML increases appetite remain to be established, but ghrelin is a potential candidate, given the negative association between ghrelin plasma concentrations and FFM previously described [22].

Therefore, the aims of this exploratory post hoc analysis were: *1*) to determine the association between %FFML during an 8-wk very low energy diet (VLED) and weight regain at 1-y follow-up, and *2*) to test the relationship between %FFML, changes in subjective feelings of appetite, and plasma concentration of appetite-related hormones during WL in a group of men and women with obesity. We hypothesized that a greater %FFML would result in *1*) greater weight regain at 1-y follow-up; and *2*) greater increases in subjective feelings of hunger and plasma concentration of the orexigenic hormone ghrelin.

Materials and Methods

Participants

Healthy adults with obesity (BMI 30–50 kg/m^2) were recruited from the local community of Trondheim, Norway by social media and articles in the local newspaper. The study was approved by the regional ethics committee (Ref. 2012/1901), registered in clinicaltrials.gov (NCT01834859), and conducted according to the Declaration of Helsinki at the Department of Clinical and Modular Medicine of the Norwegian University of Sciences and Technology. All participants signed informed consent before participation.

Participants had to be weight stable (<2 kg change over the last 3 mo), not dieting to lose weight, and with a sedentary lifestyle. Exclusion criteria were pregnancy, breast-feeding, clinically significant illness, including diabetes, previous bariatric surgery, and medication known to affect appetite/metabolism or induce WL and were based on self-reporting.

Study design

This represents an exploratory analysis using data from a longitudinal intervention study with repeated measurements, aiming at investigating compensatory mechanisms activated with a VLED and their potential relationship with WL maintenance. Briefly, participants underwent an 8-wk supervised VLED, followed by a 4-wk refeeding phase, and a 9-mo weight maintenance program. More details about the intervention can be found in previously published manuscripts [23–27].

Detailed protocol

Participants followed a VLED (Allévo, Karo Pharma AS, Stockholm, Sweden) for 8 wk (550–660 kcal/d for women and men, respectively: carbohydrates 42%, protein 36%, fat 18%, and fiber 4%), plus no-energy fluids and low-starch vegetables (maximum 100 g/d). The VLED consisted of 5 packs of a combination of soups and shakes. This was followed by a 4-wk refeeding and weight stabilization phase, in which participants were gradually reintroduced to normal food while withdrawing from the VLED products (2 VLED packs/d on week 9 and 1 pack/d on week 10). Participants were asked not to change their physical activity levels during this phase of the study.

At week 13, participants were provided with a diet plan (food-based) tailored to their individual needs (estimated from measured resting metabolic rate by indirect calorimetry × physical activity level from arm bands) and taking into account individual habits and food preferences, aiming at WL maintenance, and followed-up until 1 y. The multidisciplinary follow-up program included regular individual and group-based sessions, focusing on nutritional counseling, increased physical activity, and cognitive behavioral therapy (aimed at changing thinking and behavioral patterns) [23]. A dietitian was present in all group meetings, and participants had an individual consultation with a dietitian (1 h) every other month.

Objective measures of compliance

VLED

Participants had weekly individual 20-min consultations with a dietician throughout the 8-wk VLED period to review their food records. Urine acetoacetic acid concentration was assessed weekly using Ketostix reagent strips (Bayer Corp), as a measure of compliance. Participants who were not ketotic were educated on how to improve their compliance with the prescribed VLED. Participants were told that if they were not ketotic for more than 1 consecutive week, they would be excluded (however no participant was excluded based on this criterium).

Physical activity

Body Media (SenseWare) armband activity monitors were provided and worn for 7 d at baseline, and weeks 8 and 12. Data were considered valid if participants wore the device for ≥4 d, including at least 1 weekend day, more than 95% of the time [28].

Outcome variables

All outcome variables were measured in all participants at baseline, week 9, and 1 y. The protocol of the study was changed mid-way to also incorporate measures post-WL, when participants were out of ketosis, and in EB (week 13) in a subset of participants ($n = 35$).

All measurements were conducted after a 12-h overnight fast. Anthropometric data and body composition (FM and FFM) was collected using air-displacement plethysmography (BOD POD, COSMED). The plasma concentration of β-hydroxybutyrate (βHB), a marker of ketosis, was measured with an enzyme-linked immunosorbent assay kit (MAK-134, Sigma-Aldrich Inc).

Subjective appetite feelings [hunger, fullness, desire to eat, and prospective food consumption (PFC)] were measured with a 10-cm visual analog scale [29], and blood samples for the analysis of appetite-related hormones [active ghrelin (AG), active glucagon-like peptide 1 (GLP-1), total peptide YY (PYY), and cholecystokinin (CCK)] were collected in fasting and every 30 min after a standardized breakfast (600 kcal: 17% protein, 35% fat, and 48% carbohydrates) for 2.5 h. Plasma samples were analyzed for AG, active GLP-1, and total PYY using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit; Millipore) and for CCK using an "in-house" radioimmunoassay method as previously described [30] (intra- and interassay coefficient of variation were <10% and <20% for AG, active GLP-1, and total PYY; <10% and <15% for insulin; and <5% and <15% for CCK, respectively).

Statistical analysis

Statistical analysis was performed with SPSS version 22 (SPSS Inc), and data presented as mean ± standard deviation, unless otherwise stated. Statistical significance was set at $P < 0.05$. Participants with anthropometric data at baseline, week 9, and 1 y were included in the main analysis ($n = 72$) whereas participants with additional data at week 13 were included in the additional subanalysis ($n = 37$). All variables were assessed for normality by visual inspection of Q-Q plots and histograms, as well as normality of the residuals. There were 2 extreme values for %FFML at week 9 (+39.2% and +7.6%) that were excluded from the analysis (resulting in a sample size of 70 and 35 in the whole and subgroup analyses, respectively).

Changes in anthropometric variables, body composition, and appetite over time were assessed by either paired sample t-tests (2 timepoints), or a repeated measures ANOVA (3 or more timepoints), with Bonferroni correction for post hoc pairwise comparisons. The total area under the curve for subjective feelings of appetite and plasma concentration of appetite hormones was calculated from 0 to 150 min using the trapezoid rule.

The proportion of weight lost as FFM was calculated as the change in FFM during WL divided by total WL [i.e., %FFML = (ΔFFM/Δweight) × 100]. %FML was then 100 − %FFML. Univariate linear regressions were conducted to investigate crude associations between the predictor and the outcome variables. β-Coefficients were reported as unstandardized estimates and 95% CIs, representing the estimate and confidence of a 1-unit change in the predictor variable per 1-kg change in weight regain at 1 y (1 y–week 9 or 1 y–week 13). Next, multivariate linear regression models were generated for all individuals. Adjustments were made for the amount of WL (because WL has been shown to be a strong predictor of weight regain) [19], as well as initial FM, given that baseline body composition has been shown to modulate body composition changes with WL [31]. Multicollinearity was tested by examining the variance inflation factors of the model variables and was deemed acceptable.

Differences in %FFML, either at week 9 or week 13, between those who regained weight at 1 y and those who did not, were assessed by independent sample t-test. Moreover, potential associations between %FFML and weight regain in these 2 groups were assessed by Pearson correlation.

The association between %FFML and changes in subjective appetite feelings and plasma concentration of appetite-related hormones was investigated with Pearson correlation. If a significant association was found, univariate linear regressions were then conducted to investigate crude associations between the %FFML and the outcome variables (appetite). β-Coefficients were reported as unstandardized estimates and 95% CIs. Next, multivariate linear regression models to predict changes in ghrelin at week 9 were generated after adjusting for WL, given that basal ghrelin concentrations have been shown to be strongly inversely associated with body weight [32], and βHB, given that ketosis has been shown to prevent the increase in ghrelin secretion otherwise seen with WL [33] and an inverse association has been reported between βHB and ghrelin concentrations under ketogenic conditions [34].

Results

Anthropometrics

Seventy participants (29 males) were included in the main analysis. They had a mean age of 44 ± 9 y and a BMI of 36 ± 4 kg/m^2. Anthropometrics at baseline, week 9, and 1 y are presented in Table 1. Significant reductions in BMI, body weight,

TABLE 1
Anthropometric variables over time in all participants

	Baseline	Week 9	1 y	*P*
BMI (kg/m^2)	36.3 ± 4.0^a	30.5 ± 3.5^b	31.0 ± 4.0^b	<0.001
Weight (kg)	108 ± 17.8^a	90.9 ± 14.6^b	92.5 ± 16.6^b	<0.001
FM (kg)	47.7 ± 11.2^a	34.0 ± 10.4^b	33.4 ± 11.0^b	<0.001
FFM (kg)	60.6 ± 11.6^a	56.9 ± 10.4^b	59.1 ± 10.8^c	<0.001
%FFML		20.4 ± 10.6		
Weight regain (kg)[1]			1.73 ± 8.24	

Data presented as mean ± SD, $n = 70$. FM, fat mass; FFM, fat-free mass; %FFML, % fat-free mass loss. *P* value for main effect of time determined by repeated measures ANOVA, with Bonferroni correction for post hoc pairwise comparisons. Within a row, means without common superscript letters denote statistically significant changes overtime ($P < 0.001$ for all)

[1] Weight regain at 1 y from week 9.

and FM (kg) were seen both at week 9 and at 1 y follow-up, compared with baseline ($P < 0.001$ for all). A significant reduction in FFM was seen at week 9, followed by a significant increase from week 9 to 1 y, but 1-y values were still below baseline. %FFML at week 9 was 20.4 ± 10.6%. Mean weight regain from week 9 to 1 y was 1.7 ± 8.2 kg (8.8% ± 45.0%), with a large interindividual variation (−20.5 to 20.3 kg). Twenty-six (37 %) participants lost further weight, and 44 (63 %) regained some weight between week 9 and 1 y.

In 35 participants (24 males) with complete data at all time points (baseline, weeks 9 and 13, and 1 y), there was significant WL at week 9 followed by weight stabilization between weeks 9 and 13 (Table 2). FM (kg) was reduced at week 9, decreased further from week 9 to 13, and was maintained between week 13 and 1 y. FFM (kg) was also reduced at week 9, followed by an increase between weeks 9 and 13, and a further increase between weeks 13 and 1 y. %FFML at week 13 was 13.2% ± 11.7 %. Mean weight regain from week 13 to 1 y was 2.2 ± 7.5 kg (10.9% ± 41.3%), with a large interindividual variation (−18.2 to 22.5 kg). Twelve (34%) participants lost further weight, and 23 (66%) regained weight between week 13 and 1 y. The plasma concentration of βHB increased significantly from baseline to week 9 [0.14 ± 0.07 versus 1.32 ± 0.12 mmol/L ($n = 63$), $P <$ 0.001] and declined from week 9 to week 13 (1.35 ± 0.19 versus 0.17 ± 0.16 mmol/L ($n = 31$), $P < 0.001$, with week 13 values no longer different from baseline.

Women lost less weight, FM, and FFM than men at week 9, both in absolute (−15.2 ± 2.46 versus −20.8 ± 4.21 kg, $P <$ 0.001; −12.3 ± 2.08 versus −15.8 ± 2.94 kg FM, $P < 0.001$ and −2.89 ± 1.63 versus −4.93 ± 2.20 kg FFM, $P < 0.001$) and relative terms (−15.1 ± 1.55 versus −17.4 ± 2.46%, $P < 0.001$ for WL; −6.05 ± 1.86% versus −8.00 ± 2.58%, $P < 0.001$ for FM; and −5.98±1.79 versus −7.73±2.67%, $P < 0.01$ for FFM). However, weight regain did not differ (0.65 ± 6.44 versus 3.17 ± 9.92 kg, $P = 0.20$; 5.43 ± 44.4 versus 14.1 ± 45.4%, $P = 0.425$) and no differences in %FFML were seen between sexes, even though there was a trend for women to lose less FFM (−18.4/12.0 versus −23.2/7.57%, $P = 0.063$). Similar differences were observed at week 13, despite lower significance values.

TABLE 2
Anthropometric variables over time in a subset of participants with data at all timepoints

	Baseline	Week 9	Week 13	1 y	*P*
BMI (kg/m^2)	36.6 ± 4.4^a	30.5 ± 3.8^b	30.3 ± 3.9^b	31.0 ± 4.6^b	<0.001
Weight (kg)	116 ± 18.8^a	96.4 ± 15.4^b	95.7 ± 15.8^b	98.1 ± 19.1^b	<0.001
FM (kg)	48.4 ± 12.8^a	33.8 ± 12.3^b	30.6 ± 13.0^c	32.8 ± 12.8^{bc}	<0.001
FFM (kg)	67.1 ± 11.6^a	62.9 ± 10.0^b	64.2 ± 10.3^d	65.3 ± 10.5^e	<0.001
%FFML		21.1 ± 8.2	13.2 ± 11.7		
Weight regain (kg)[1]				2.31 ± 9.02	

Data presented as mean ± SD, $n = 35$. FM, fat mass; FFM, fat-free mass; %FFML, % fat-free mass loss. *P* value for main effect of time determined by repeated measures ANOVA, with Bonferroni correction for post hoc pairwise comparisons. Within a row, means without a common superscript letter denote statistically significant differences: ^{a,b,c} $P <$ 0.001; ^{d,e} $P < 0.05$.

[1] Weight regain at 1 y from week 13.

Compliance

VLED

Compliance with the VLED was excellent based on measurements of acetoacetate in urine (all participants were ketotic). Compliance was defined as no negative readings of acetoacetic acid concentration in the urine.

Physical activity

No change in physical activity over time was recorded (data not shown).

Association between %FFML and weight regain

Table 3 provides univariate regression results predicting weight regain from week 9 to 1 y. FM (%) at baseline significantly predicted weight regain, whereas FFM (kg) [but not FM (kg)] tended toward a significant association. %FFML at week 9 was found not to be a significant predictor of weight regain at 1 y. Similar results were obtained when trying to predict weight regain from week 13 to 1 y, using either baseline data or changes in body weight/composition from baseline to week 13 ($n = 35$). Also, analyzing men and women separately yielded similar outcomes (data not shown).

Table 4 reports results from a multivariate linear model. Baseline FM (kg) and WL were significant predictors of weight regain at 1-y follow-up, but not %FFML. When trying to predict weight regain from week 13 to 1 y using the same variables, no significant predictor was found, and the model was not significant. Also, analyzing men and women separately yielded similar outcomes (data not shown).

There were no significant differences in %FFML between those who regained weight at 1 y versus those who did not, either at week 9 or week 13 (Table 5). However, a trend toward an association between %FFML at week 13 and weight regain at 1 y (from week 13) was seen in those who regained weight.

Changes in appetite

Subjective feelings of appetite at baseline and week 9 can be seen in Supplemental Table 1. There was a significant increase in hunger and decrease in PFC in the fasting state ($P = 0.025$ and $P = 0.002$, respectively). There was also a decrease in hunger,

TABLE 3
Univariate regression analysis predicting weight regain at 1 y

Predictor	β (95% CI)	R^2	*P*
Age	−0.05 (−0.26, 0.16)	0.01	0.63
Sex	2.41 (−1.53, 6.35)	0.02	0.23
Baseline weight (kg)	0.01 (−0.10, 0.12)	0	0.88
Baseline FFM (kg)	0.15 (−0.02, 0.31)	0.044	0.08
Baseline FM (kg)	−0.14 (−0.31, 0.03)	0.037	0.11
Baseline FM (%)	−0.33 (−0.63, −0.03)	0.05	0.03
Weight loss (kg)	0.29 (−0.17, 0.75)	0.02	0.21
FM loss (kg)	−0.50 (−1.15, 0.15)	0.03	0.13
FFM loss (kg)	−0.03 (−0.96, 0.90)	0	0.90
%FFML week 9	0.04 (−0.15, 0.22)	0.01	0.70

Univariate linear regression analyses predicting weight regain at 1 y (from week 9), $n = 70$. Each unstandardized β-coefficient represents 1 kg weight regain at 1 y per unit of the predictor. FM, fat mass; FFM, fat-free mass; %FFML, fat-free mass loss.

TABLE 4
Multivariate linear regression models predicting weight regain at 1 y

Predictor	β (95% CI)	*P*	Adjusted R^2
Multivariate model	4.70 (−6.6, 15.9)	0.05	0.07
Constant	0.61 (0.08, 1.13)	0.41	
Weight loss (kg)		0.02	
Baseline FM (kg)	−0.22 (−0.41, -0.04)	0.02	
%FFML week 9	0.14 (−0.15, 0.43)	0.33	

Multivariate linear regression analyses predicting weight regain at 1 y (from week 9). Each unstandardized β-coefficient represents 1 kg weight regain at 1 y per unit of the predictor. FM, fat mass; %FFML, fat-free mass loss. Variance inflation factors <1.4.

TABLE 5
%FFML in those who regained or lost further weight at 1 y follow-up

	Regained (n = 44/23)[1]	Lost further weight (n = 26/12)[1]	*P*
%FFML week 9	−19.8 ± 11.9	−21.4 ± 8.0	0.56
%FFML week 13	−12.4 ± 8.6	−14.7 ± 16.4	0.66

P value for differences between groups assessed by independent sample t-test.
[1] Sample size at weeks 9 and 13, respectively; %FFML: % fat-free mass loss.

desire to eat, and PFC in the postprandial state ($P = 0.049$, $P = 0.015$, and $P < 0.001$, respectively), and an increase in postprandial fullness ($P < 0.001$). Plasma concentration of appetite hormones at baseline and week 9 can be seen in Supplemental Table 2. No significant changes were seen on ghrelin concentrations. There was a significant reduction in basal GLP-1 ($P = 0.01$), but an increase in postprandial GLP-1 ($P < 0.001$). Basal and postprandial concentration of CCK were significantly reduced at week 9 ($P = 0.001$ and $P < 0.001$, respectively).

Subjective feelings of appetite and plasma concentrations of appetite-related hormones at baseline, week 9, and week 13 in a subset of the sample ($n = 35$) can be seen in Supplemental Tables 3 and 4. No significant change in hunger feelings in the fasting state was seen at week 9, but there was an increase from week 9 to 13 ($P = 0.01$), with ratings at week 13 significantly higher than baseline ($P = 0.004$). Moreover, a significant increase in desire to eat in fasting was seen between weeks 9 and 13 ($P = 0.013$). No significant changes in basal ghrelin concentrations were see between baseline and week 9, followed by an increase between weeks 9 and 13, so that week 13 concentrations were above baseline ($P < 0.001$ for both). Postprandial concentrations of ghrelin increased from baseline to week 9 ($P < 0.05$), followed by a further increase between weeks 9 and 13 ($P < 0.01$). Basal active GLP-1 increased from week 9 to 13 ($P < 0.05$), whereas postprandial concentrations decreased during the same period ($P < 0.01$). Basal concentrations of total PYY decreased between baseline and week 9 and increased thereafter ($P < 0.05$ for both). Basal and postprandial concentrations of CCK decreased from baseline to week 9 ($P < 0.01$ and $P < 0.001$, respectively), followed by an increase from week 9 to 13 ($P < 0.05$ and $P < 0.001$, respectively), with no differences between week 13 and baseline.

Association between %FFML and appetite

A significant association was seen between %FFML at week 9 and changes in basal ghrelin concentrations during the same period (Figure). The larger the %FFML at week 9, the smaller the reduction (in those who experienced a reduction in ghrelin concentrations) or greater the increase in basal ghrelin concentration (in those who experienced an increase in ghrelin concentration) (R^2 adj = 13%, $P = 0.002$). This association was maintained even after adjustment for WL (kg) and βHB (Table 6). No associations were seen between %FFML at week 9 and changes in other appetite variables nor between %FFML at week 13 and changes in either subjective appetite feelings or plasma concentration of appetite-related hormones.

Discussion

The present exploratory analysis aimed to determine the association between %FFML during WL induced by a VLED and weight regain at 1 y and to test the relationship between %FFML and changes in appetite variables in a group of men and women with obesity. These analyses were performed both when participants were ketotic and in negative EB (week 9), and outside of ketosis and in EB (week 13). Ketosis was defined as the presence of acetoacetate (a ketone body) in the urine. %FFML, either at week 9 or 13, was not associated with weight regain. However, at week 9, the greater the %FFML, the smaller the reduction, or greater the increase, in basal ghrelin plasma concentration over time.

Several reasons may contribute to the negative findings regarding %FFML and weight regain in the present analyses, namely the baseline characteristics of the participants and the nature of the intervention. Our participants were much heavier (mean BMI: 36 ± 4 kg/m^2, weight: 108.4 ± 17.8 kg) than in the Vink et al. study (mean BMI: 31 kg/m^2) [18] and Turicchi et al. (weight: 99.6 ± 16.3 kg) study [20]. Fat overshooting is likely to be weaker as BMI and FM increase, given that the proportion of weight lost as FFM decreases as BMI and FM increase [35]. Moreover, in our analysis, men corresponded to only 41% of the sample but were 90% in the Vink et al. study [18], and in the Turicchi et al. study [20], the association between %FFML and weight regain was only seen in men. The association between %FFML and weight regain might be stronger in men due to sex differences in body composition. Men have a lower FM than women and are, therefore, more prone to lose FFM with WL interventions [36]. Mean weight regain in the present study [1.7 kg (9%) from week 9 to 1 y, and 2.3 kg (11%) from week 13 to 1 y] was smaller compared with the Vink et al. study [4.2 kg (59%) in the low-energy diet (LED)-group and 4.5 kg (55%) in the very-low energy diet (VLED)-group] [18]. In Turicchi et al. [20], participants regained 1.6 kg (14%), and men 3.0 kg (23%) between weeks 8 and 26. It might be that the higher baseline BMI of our participants, the fact that the majority was female, and that mean weight regain at 1-y follow-up was relatively low contributed to the negative findings regarding %FFML and weight regain. Analysis in men did not reveal an association between %FFML and weight regain in the present analysis, potentially due to lack of power ($n = 25$).

There was a tendency for a larger %FFML at week 13 to be associated with a greater weight regain in those who regained weight. This finding is interesting, as those who can further

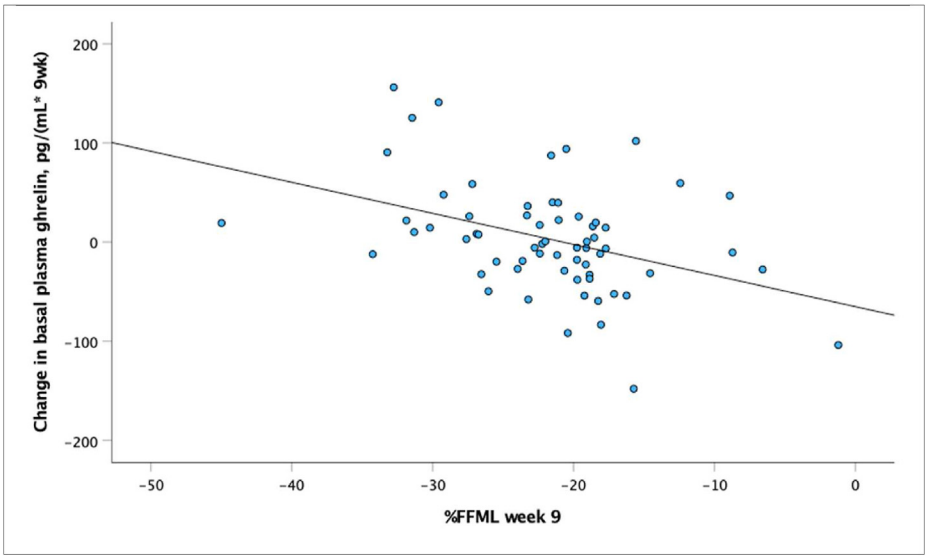

FIGURE. Scatterplot for the association between the proportion of weight lost as fat-free mass (%FFML) during the 8-wk very low energy diet and changes in basal ghrelin plasma concentration during the same period.

TABLE 6
Multivariate linear regression model to predict change in basal ghrelin at week 9

Predictor	β (95% CI)	*P*	Adjusted R^2
Multivariate model	−40.3 (−109, 28.4)	0.01	0.15
Constant		0.25	
Weight loss (kg)	−0.21 (−3.5, 3.1)	0.90	
βHB (mM)	−14.2 (−30.3, 1.9)	0.08	
%FFML week 9	−3.2 (−5.0, −1.1)	0.01	

Multivariate linear regression analyses predicting changes in basal ghrelin from baseline to week 9, n = 70. βHB, β-hydroxybutyrate; % FFML, % fat-free mass loss. Variance inflation factors (VIF) <1.1.

reduce their body weight after a WL of 20 kg likely represent a very unique group of highly motivated individuals, in which behavior clearly overrides any potential weak physiological drivers of relapse. Indeed, in our recent study in premenopausal women, a greater %FFML was associated with more weight regain at 1-y follow-up and most individuals regained weight (range: −3.6 to 20.3 kg, mean 6.0 ± 4.4 kg or 51.3 ± 37.8%) [17]. This is very different from the present study where mean weight regain was minimal and only slightly more than half of the individuals regained weight. The lack of statistical significance is likely explained by lack of power, as the sample size was small ($n = 23$). The fact that this association was not seen at week 9 may derive from the limitations associated with the method of measuring body composition (discussed in detail below) and/or differences in sex distribution, as males represented 41% of the sample at week 13 but 69% at week 9. Sex has long been recognized as an important variable modulating the %FFML in response to energy-restricted diets, being much larger in men than in women [37], as also observed in this study.

In line with previous findings [38,39], including our own [24,27], we observed that ketosis minimized or prevented the increase in basal plasma concentration of ghrelin, the only peripheral hormone with orexigenic properties, and hunger feelings, otherwise seen with WL [24,27,38,39]. Interestingly, we found that the greater the %FFML at week 9, the smaller the reduction, or greater the increase, in basal ghrelin plasma concentration, even after adjusting for WL and βHB. This increased orexigenic response with greater %FFML is in line with Turicchi et al. [20] who reported that in men a larger %FFML was associated with a larger increase in postprandial hunger and desire to eat.

The exact mechanisms through which %FFML modulates appetite remain to be fully elucidated, but it has been suggested that signals released from the muscle, referred to as myokines, during FFM loss may act at the level of the brain and modulate appetite control [13,40,41]. For example, central irisin infusion has been reported to decrease serum leptin and increase ghrelin concentrations, with an overall increase in food intake in rats [12]. Moreover, ghrelin has been implicated in myocyte growth [42–44]. As such, it has been suggested that FFM loss following energy restriction might lead to the activation of the preproghrelin gene and ghrelin, which would then act on the myocyte to increase myoblast differentiation [41]. Additionally, FFM might also modulate the hedonic appetite control system. Flack et al. [45] showed that in young adults with overweight/obesity, that those who lost the greatest amount of FFM in response to a 12-wk aerobic exercise intervention were also the ones who experienced the highest increase in reward-driven feeding (using a behavioral choice task), even after adjusting for changes in FM and other potential confounders [45].

Surprisingly the association between %FFML and changes in ghrelin plasma concentrations disappeared at week 13 when participants were no longer ketotic. The association between % FFML and changes in hunger and desire to eat in the Turicchi et al. study were likely also observed in ketosis, as the measurements of appetite were done immediately after an 8-wk 800 kcal/d diet. Weight loss in the present study was induced by a VLED, and analysis of βHB plasma concentrations (a marker of ketosis) showed that participants were ketotic at week 9 but not at week 13. Ketosis is accompanied by glycogen depletion and with it water loss, whereas refeeding is followed by glycogen replenishment and with it increased water content [46,47]. We used a 2-compartment model air displacement plephysmography

(ADP) to measure body composition, whereas Turicchi et al. used dual energy x-ray absorptiometry (DXA) [20]. Both methods of body composition fail to account for changes in the hydration of lean tissue. This might explain why we saw a significant increase in FFM between weeks 9 and 13, despite weight maintenance. Losses of FFM might be exaggerated when measurements are done under negative EB, particularly when in ketogenic conditions, as loss of body water due to glycogen depletion is not accounted for. The exact reasons for why we found an association between %FFML and ghrelin only under ketogenic conditions remains to be explored, but ketosis is unlikely to be involved, as the association was seen even after adjusting for βHB. Alternatively, it might be related to the fact that participants were in active WL at week 9, but were weight stable at week 13, or to lack of power at week 13.

This study has both strengths and limitations. A strength of this study is that it included measurements of body composition and appetite both in and out of ketosis, allowing for the separation of the effect of negative EB and ketosis (week 9) from the independent effect of WL (week 13). However, body composition was done with a 2-compartment model and did not account for changes in the hydration of the lean tissue. This might have compromised the validity of the results at week 9. Second, despite a large initial WL (mean 20 kg), weight regain at 1-y follow-up was minimal (mean 1.7 kg), which is atypical and might have compromised the identification of predictors of weight regain. Third, a multiplex assay was employed for the measurements of appetite hormones (except for CCK), which is likely to result in less accurate and precise measurements compared with optimized assays for each individual hormone. Fourth, even though we used Bonferroni correction to account for multiple comparisons, we did not adjust for the large number of statistical tests, which could inflate type 1 error rate. Finally, because this is an exploratory analysis, it is likely underpowered to examine the association between %FFML and weight regain and especially the association between %FFML and changes in a large number of appetite markers. More studies with larger sample sizes, a balanced sex distribution that allows for the investigation of potential important sex interactions, and a large range of BMI and FM are needed. A larger sample size might be especially important as FFM has been shown to account for only 4.8% of the total variance regarding weight regain [20]. Additionally, further research on the role of functional body composition changes during WL on weight regain should use advanced methods and multicompartment models to provide additional mechanistic insights.

In conclusion, %FFML does not seem to be a significant predictor of weight regain at 1 y in a mixed sample of men and women with obesity. However, larger studies with a greater initial loss of FFM and long-term weight regain are needed to clearly establish the role of %FFML on relapse in obesity management.

Acknowledgments

We thank all participants for their time and commitment, Hege Bjøru and Sissel Salater (at the ObeCe, Clinic of Surgery, St. Olav University Hospital) for support with screening and blood collection, and Ingrid Hals (at the Department of Clinical and Molecular Medicine, NTNU) for support with lab work. The authors' responsibilities were as follows—CM, SN: designed the study; SN: collected the data; SN, SRC: measured all the appetite hormones, with the exception of CCK that was analyzed in JFR's lab; and all authors: contributed to manuscript writing and approved the final version the manuscript.

Funding

This work was supported by the Liaison Committee for education, research and innovation in Central Norway. Allevò, Karo Pharma AS (Stockholm, Sweden), provided the very low energy diet products (no commercial interest). The sponsors had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Author disclosures

The authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.03.026.

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