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Randomized Controlled Trial

Gastrointestinal hormones and subjective ratings of appetite after low-carbohydrate vs low-fat low-energy diets in females with lipedema – A randomized controlled trial

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SUMMARY

Background: Ketosis seems to attenuate, or prevent, the rise in both ghrelin concentrations and subjective hunger ratings that follow weight loss. However, most of the previous studies have employed very-low energy diets (VLED) and are therefore limited in terms of generalizability.
Objectives: To compare changes in ghrelin plasma concentrations after a low-carbohydrate (LCD) versus an isocaloric low-fat low energy diet (LED) in females with lipedema. Secondary objectives were to determine potential differences between diets in changes in satiety hormones, and subjective ratings of appetite.
Methods: Females with obesity and lipedema were randomized to either an LCD (75 g carbohydrates) or low-fat diet (180 g carbohydrates) for 8 weeks. Plasma concentrations of ghrelin, peptide YY, cholecystokinin (CCK), and glucagon-like peptide 1 (GLP-1), and subjective ratings of appetite were measured in the fasting and postprandial states, pre and post intervention.
Results: 55 females (30 in LCD) were included (age 47.9 ± 11.3 years, BMI 36.8 ± 5.1 kg/m^2). Both LCD and low-fat groups lost weight (10.3 %, $P < 0.001$ and 7.3 %, $P < 0.001$, respectively), but the LCD lost significantly more. No within or between groups differences were found for ghrelin in the fasting state. A reduction in postprandial (tAUC) ghrelin was seen only in the LCD group ($P = 0.002$), and this change was significantly different from the low-fat group ($P = 0.046$). The LCD group also reported an increase in postprandial (both iAUC and tAUC) fullness ratings ($P = 0.035$ and $P = 0.005$, respectively), but this was not significantly different from the low-fat group ($P = 0.703$ and $P = 0.365$, respectively), despite the latter experiencing no change ($P = 0.127$ and $P = 0.152$, respectively). Conversely, only the low-fat group reported increased hunger in fasting ($P = 0.046$), but changes were not significantly different from the LCD group ($P = 0.711$). A decrease in postprandial (both tAUC and iAUC) CCK was observed in both LCD and low-fat diet groups ($P \leq 0.005$ for all).
Conclusion: Despite no changes in fasting ghrelin concentrations in either of the diet groups, a reduction in postprandial ghrelin and increased fullness was seen in the LCD group. These favorable changes in appetite in the LCD group might have contributed to the greater weight loss observed in this group.

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Clinical trial registration: NCT04632810, Effect of Ketosis on Pain and Quality of Life in Patients With Lipedema (Lipodiet).

Abbreviations

AcAc	Acetoacetate
CCK	Cholecystokinin
CHO	Carbohydrate
DTE	Desire to eat
E%	Energy percentage
FFM	fat free mass
FM	Fat mass
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
iAUC	Incremental area under the curve
LCD	Low-carbohydrate diet
LED	Low-energy diet
MET	Metabolic equivalent of task
MVPA	Moderate to vigorous physical activity
PFC	Prospective food consumption
PYY	Peptide YY
REK	Regional ethical committee
βHB	β-hydroxybutyrate
tAUC	Total incremental area under the curve
VLED	Very low-energy diet
W9	Week 9

1. Introduction

Lipedema is an underdiagnosed disease characterized by subcutaneous adipose tissue accumulation [1] in the lower extremities [2], and with pain in the affected areas [3]. Low-carbohydrate (CHO) diets (LCD) and very-low carbohydrate ketogenic diets have been proposed as a good treatment option for females with lipedema [4], due to their benefit in reducing pain [5–8], improving quality of life [5,8] and reducing body weight and fat mass (FM) [9]. Weight gain seems to aggravate lipedema [10], hence weight management is crucial in this patient group [11]. However, diet-induced weight loss has consistently been shown to increase the concentrations of the hunger hormone ghrelin [12–15], as well as subjective ratings of hunger [12,14,15]. This might contribute to poor adherence to energy restricted diets, reduced compliance to dietary interventions, and suboptimal weight loss [16].

Ketosis is a metabolic state that occurs when CHO intake falls below a certain threshold, and a shift in energy metabolism occurs, from CHO to fat oxidation [17]. Ketosis seems to attenuate, or prevent, the rise in both ghrelin concentrations and subjective ratings of hunger that follow weight loss [14,15,18,19]. However, its effects on the plasma concentrations of satiety peptides, namely glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and cholecystokinin (CCK), as well as subjective ratings of fullness, remain controversial [14,15,18,19]. Additionally, most of the previous studies have employed very low-energy diets (VLEDs) [12,14,15,19], and are therefore limited in terms of generalizability. Nutritional-induced ketosis can be achieved not only with VLEDs, but also fasting, LCD, and very-low CHO ketogenic diets [17]. Despite a lack of consensus, a very-low CHO is usually defined as a diet providing <10 energy percent (E%) from CHO, and a LCD as providing between 10 and 25 E% from CHO [17]. VLEDs usually provide less than 800 kcal and 40–60 g CHO/day [20].

Therefore, the primary objective of this study was to compare changes in fasting ghrelin plasma concentrations after 8 weeks on two isocaloric low-energy diets (LED): LCD versus low-fat diet in females with lipedema. Secondary objectives were to determine potential differences in the changes in fasting and postprandial plasma concentrations of total GLP-1, PYY, CCK, as well as subjective ratings of appetite between the two diet groups.

2. Methods

2.1. Study design

This manuscript describes secondary analyses of a randomized controlled trial (RCT) comparing the effects of an LCD versus a low-fat LED on pain and quality of life (QoL) in females with lipedema [8]. The study was approved by the Regional Ethical Committee of Central Norway (REK 93888) and registered in Clinicaltrials. gov (NCT04632810). All participants provided written informed consent in line with the Helsinki Declaration, before entering the study.

Participants were randomized (1:1) by block randomization with stratification by body mass index (BMI) categories (30.0–34.9, 35.0–39.9, 40.0–44.9 kg/m^2). Randomization was performed by a web-based randomization system developed and administered by the faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway. Data collection was done with eFORSK, a web-based system developed and administered by Helse Midt-Norge IT (Central Norway Regional Health Authority's IT department).

2.2. Study population

Females diagnosed with lipedema and obesity (BMI between 30 and 45 kg/m^2), aged 18–75 years old, and weight stable for the last three months (±3 kg) were invited to participate in the study. Participants were diagnosed with lipedema by physiotherapists before inclusion, and type and stage of lipedema were assessed at baseline by study personnel [8,16]. Exclusion criteria included acute and chronic kidney disease/failure, previous bariatric surgery, malignant disease, infectious disease, diabetes, psychosocial disorders, breastfeeding, pregnancy, current medication known to affect body weight and/or appetite, not mastering a Scandinavian language, and enrollment in another obesity/lipedema treatment program (except from regular physiotherapy, including compression garments/pulsator, etc.).

2.3. Intervention

The intervention was run at the Obesity out-patient clinic at St. Olavs' University hospital (Trondheim, Norway) from June 2021 to

May 2023. Diets were matched for energy (1200 kcal/day) and protein (60 g, energy percentage (E%) 20 E%) but differed in CHO and fat content. The LCD consisted of 75 g of CHO (25 E%) and 73 g of fat (55 E%), while the low-fat diet consisted of 180 g of CHO (60 E %) and 27 g of fat (20 E%). The dietary plans were adjusted with respect to food preferences, intolerances, and allergies by dietitians. Participants were advised to take a multivitamin-mineral supplement and to drink a minimum of two liters of calorie-free drinks daily (limiting artificially sweetened beverages). Participants were asked not to change their physical activity levels throughout the study period.

2.4. Compliance

Participants had weekly follow-ups (see Fig 1), either by phone or face-to-face, depending on convenience. Body weight was measured, ketosis assessed (see below) and potential side effects of the diets discussed, aiming at enhancing compliance and preventing dropouts. Necessary changes and adjustments in the diets were made, within the constraints of energy and macronutrient distribution. Participants were asked to fill out daily food records throughout the whole intervention period, where they reported which pre-specified meal they ate, and if there were any deviation from this meal. These were then analyzed for intake of energy (kcal/day) and macronutrients (g/day, E %), using a web-based analysis program [21] based on the Norwegian Food Composition Table [22], and discussed at the weekly follow-ups.

Ketostix® reagent test strips (Ascensia Diabetes Care Holdings AG, Basel, Switzerland) were used in the weekly follow-ups to measure urinary acetoacetate (AcAc) concentration. A cutoff level <0.5 mmol/L was used for negative AcAc. β-hydroxybutyrate (βHB) concentration was also measured in plasma samples using an ELISA-kit (MAK134, Sigma–Aldrich Inc.) at baseline and week 9 (w9) and in whole blood using finger-pricks (Freestyle Precision Neo, Abbott, CA, USA) and Freestyle β-ketone reagent strips (Abbott, CA, USA) at baseline, w5 and w9. Participants with a βHB plasma concentration ≥0.3 mmol/L at week 9, were defined as being in nutritional-induced ketosis. Participants in the LCD group had to be ketotic at w9, while participants in the low-fat group had to be non-ketotic based on βHB data to be included in the analyses. If borderline at w9, ≥4 AcAc urine concentrations in line with group allocation during the weekly follow-ups, were needed for inclusion in the analysis.

Physical activity was assessed using accelerometers (Actigraph wGT3X-BT) for 7 days prior to baseline, at week 4 (w4) and the last week of the study (week 8). Data were considered valid if the device was worn for ≥4 days, with ≥95 % (22.8 h of the day) wear time [23]. Average metabolic equivalent of task (MET), time in sedentary, light, and moderate to vigorous (MVPA) physical activity were included.

2.5. Outcome variables

The following variables were measured at baseline and Wk9.

2.5.1. Body weight and body composition

Body weight was measured wearing only underwear with Seca 876 (SECA, Hamburg, Germany) to the nearest 0.1 kg. Body composition (FM and fat free mass (FFM)) was measured using air-displacement plethysmography (BodPod, COSMED, Rome, Italy) in the fasting state (>8 h).

2.5.2. Appetite

Plasma concentration of gastrointestinal (GI) hormones and subjective appetite ratings were assessed in fasting and after a standardized breakfast. The standardized breakfast consisted of oat bread, butter, strawberry jam, cheese, orange juice, and either milk or yogurt, with approximately 600 kcal (17 % protein, 35 % fat and 48 % CHO) and was consumed in the lab at our faculty for 15 min.

Blood samples were collected in the fasting state, and every 30 min after the standardized breakfast until 150 min. For analysis of acylated ghrelin, and total PYY, one milliliter of whole blood was transferred into a microtube and a 20 μl mixture of inhibitor (10 μl of Pefabloc protease inhibitor (Roche Diagnostic, Germany) and 10 μl dipeptidyl-peptidase IV inhibitor (Merck Millipore, Germany)) was added. For CCK and total GLP-1, 500 KIU of aprotinin (DSM, Coatech AB) per milliliter of whole blood was added to the EDTA tubes. Samples were then centrifuged at 3200 RPM, 18 °C for 10 min, and the plasma frozen at −80 °C until further analysis. Samples for acylated ghrelin and total PYY were analyzed using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore, St. Louis, MO, USA). CCK and total GLP-1 were analyzed using validated "in-house" radioimmunoassay (RIA) methods [24,25]. Intra- and inter-assay coefficient of variation (%CV) were <17.7 and < 12 for acylated ghrelin, <5 and < 15 for total GLP-1, <18.2 and < 20 for PYY, and <5 and < 15 for CCK, respectively.

Subjective ratings of appetite (hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC)) were assessed using a 100 mm visual analogue scale (VAS) [26] in fasting, immediately after the standardized breakfast, and every 30 min until 150 min. The end of the lines represents the most extreme feelings you've ever felt, "I have never been more hungry" etc.

2.6. Statistical analysis

Statistical analysis was performed using Stata 18 (StataCorp LLC, Texas, USA), data presented as means ± SD, and statistical significance set at $P < 0.05$. Total and incremental area under the curve (tAUC and iAUC) were calculated from 0 to 150 min using the trapezoid rule. Group differences in the changes from baseline in all outcome variables (fasting values, tAUC and iAUC) were estimated

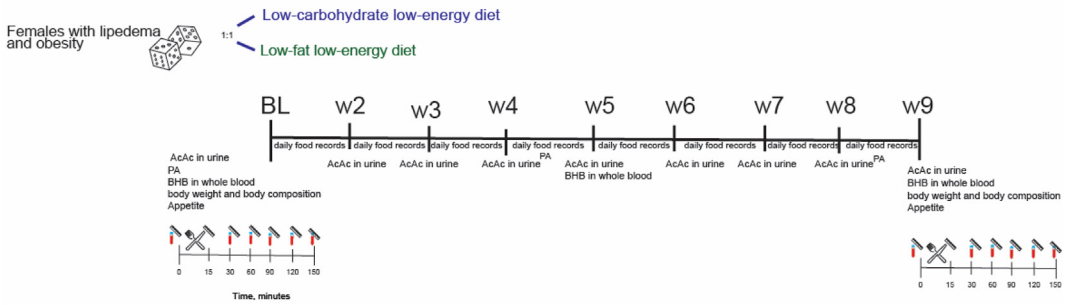

Fig. 1. Overview of study design and outcome variables. BL: Baseline. W: Week. PA: Physical activity. BHB: Beta-hydroxybutyrate. AcAc: Acetoacetate.

Table 1
Baseline characteristics of the participants.

	All participants (n = 55)	LCD (n = 30)	Low-fat diet (n = 25)
	Mean ± SD	Mean ± SD	Mean ± SD
Age, years	47.9 ± 11.3	48.5 ± 8.6	47.3 ± 14.0
Height, cm	166.4 ± 6.1	166.6 ± 6.9	166.3 ± 5.2
BMI, kg/m^2	36.8 ± 5.1	36.7 ± 4.6	37.0 ± 5.8
Weight, kg	102.0 ± 14.7	101.7 ± 13.5	102.4 ± 16.3
Lipedema type			
1, *n* (%)	4 (7.1 %)	2 (6.7 %)	2 (7.7 %)
1 + 4, *n* (%)	1 (1.8 %)	0 (0 %)	0 (0 %)
2, *n* (%)	9 (16.1 %)	6 (20 %)	3 (11.5 %)
2 + 4, *n* (%)	1 (1.8 %)	1 (3.3 %)	0 (0 %)
3, *n* (%)	12 (21.4 %)	3 (10 %)	9 (34.6 %)
3 + 4, *n* (%)	22 (39.3 %)	14 (46.7 %)	8 (30.8 %)
4, *n* (%)	1 (1.8 %)	1 (3.3 %)	0 (0 %)
5, *n* (%)	6 (10.7 %)	2 (6.7 %)	4 (15.4 %)
Lipedema stage			
1, *n* (%)	10 (17.9 %)	5 (16.7 %)	5 (19 %)
2, *n* (%)	38 (67.9 %)	20 (66.7 %)	8 (30.8 %)
3, *n* (%)	8 (14.3 %)	5 (16.7 %)	3 (11.5 %)

Data presented as mean ± SD and numbers (percentage). BMI: body mass index. LCD: low-carbohydrate low-energy diet. Low-fat diet: low-fat low-energy diet. Type 1: pelvis, buttocks, and hips. Type 2: buttocks to knees. Type 3: buttocks to ankles. Type 4: arms. Type 5: lower legs. Stage 1: normal and smooth skin surface. Stage 2: uneven skin where indentions of fat is visible. Stage 3: expansions of tissue causing deformations, most common on the thigs and around knees [2,29].

by linear mixed-effect models. The fixed part was specified in terms of two dummy variables: one for time (within low-fat diet group) and one for group differences (LCD vs. low-fat diet) post intervention (week 9), since baseline means can be assumed to be the same given the randomized design of the study [27,28]. The mean difference in changes from baseline is equivalent to the estimated mean group difference post intervention (between groups). Linear combination of the parameters in the models were assessed to estimates changes in the LCD group. Associations between βHB and gastrointestinal hormones and subjective appetite sensations were done using linear regression. A random intercept for patient was included to account for within-patient correlations. Residuals were checked for normality using histograms and Shapiro wilk-test. Figures were generated using GraphPad Prism (Version 10.0.2 for Windows, GraphPad Software, Boston, Massachusetts, USA).

3. Results

3.1. Participant characteristics

General characteristics of the participants are presented in Table 1. A total of 55 females with an average BMI of 36.8 ± 5.1 kg/m^2 and age of 47.9 ± 11.3 years were included in this analysis (see Fig. 2). Five dropouts were seen in the low-fat diet group and 3 drop-outs in the LCD.

3.2. Compliance

Participants' daily energy and macronutrient intake is presented in Supplementary Table 1, and βHB plasma concentrations at baseline and w9 and weekly AcAc urine concentrations are presented in Table 2. No differences between groups were found for energy intake (P = 0.650). However, the LCD group had a higher fat and protein intake, and a lower fiber and CHO intake compared to the low-fat group (all P < 0.001).

Participants' physical activity levels are presented in Supplementary Table 2. Both groups had a significant increase in sedentary time (P = 0.022 and P = 0.009, for LCD and low-fat, respectively) and a decrease in light activity duration (P < 0.001

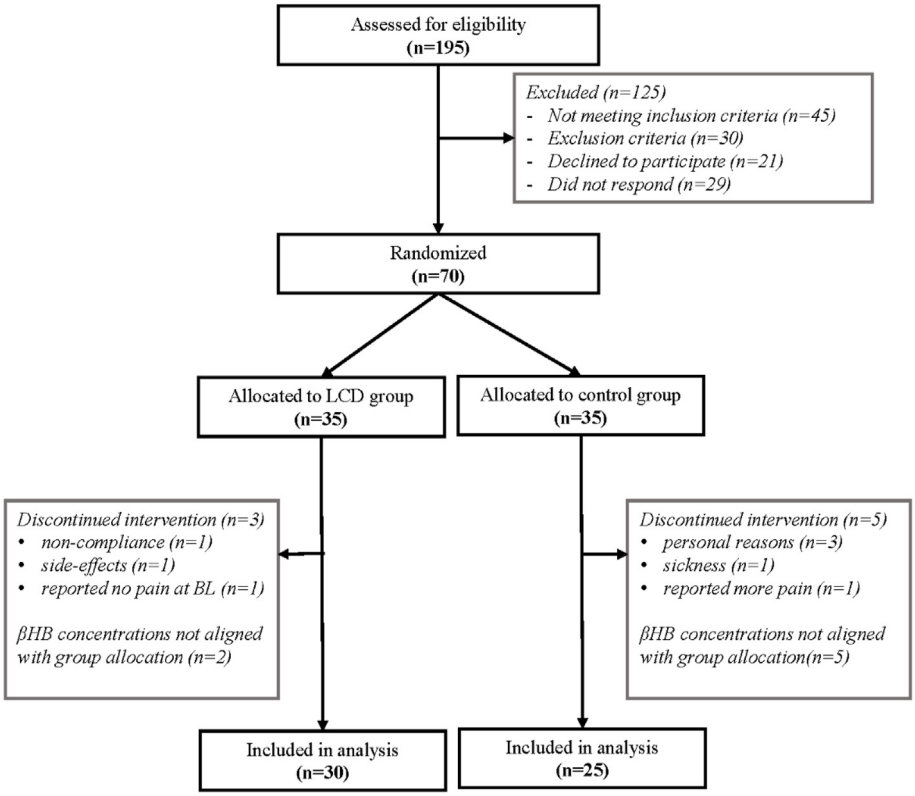

Fig. 2. Flow chart of the study. LCD: Low-carbohydrate diet. BL: Baseline. βHB: β-hydroxybutyrate.

Table 2
Concentrations of acetoacetate and β-hydroxybutyrate over time.

	AcAc in urine (mmol/L)		βHB in plasma (mmol/L)		βHB in whole blood (mmol/L)	
	LCD (n = 30)	Low-fat diet (n = 25)	LCD (n = 30)	Low-fat diet (n = 25)	LCD (n = 30)	Low-fat diet (n = 25)
Baseline	0.0 ± 0.1	0.0 ± 0.0	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
Week 2	2.4 ± 2.3[a]	0.4 ± 0.9				
Week 3	2.9 ± 2.2[a]	0.1 ± 0.3				
Week 4	2.7 ± 2.3[a]	0.4 ± 0.8				
Week 5	2.3 ± 2.5[a]	0.1 ± 0.3			0.7 ± 0.4[a]	0.1 ± 0.1
Week 6	2.4 ± 2.4[a]	0.1 ± 0.2				
Week 7	1.9 ± 1.8[a]	0.0 ± 0.1				
Week 8	2.1 ± 2.2[a]	0.2 ± 0.5				
Week 9	1.8 ± 2.4[a]	0.0 ± 0.1	0.8 ± 0.4[a]	0.1 ± 0.1	0.7 ± 0.5[a]	0.2 ± 0.1

Data presented as mean ± SD. BL: baseline. LCD: low carbohydrate diet. βHB: beta-hydroxybutyrate. AcAc: acetoacetate.
[a] Significant different from low-fat diet group. P < 0.05.

for both) from baseline to w9. No differences between groups were found.

3.3. Body weight and composition

Changes in body weight and composition are presented in Table 3. Both the LCD and low-fat diet groups experienced a significant reduction in body weight (10.3 vs 7.3 %, respectively), FM in both absolute values and percentage, and FFM. The reduction in body weight and FM (kg and %) was greater in the LCD group compared to the low-fat diet group.

3.4. Gastrointestinal hormones

Fasting and postprandial plasma concentrations of GI hormones at baseline and w9 are presented in Fig. 3 and Supplementary Table 3. No changes in fasting ghrelin concentrations were seen in either the LCD (0.7 pmol/L, 95 % CI: −9.7 to 11.2, P = 0.890, Fig. 3A) or the low-fat group (4.3 pmol/L, 95 % CI: −7.2 to 15.9, P = 0.462, Fig. 3A), with no significant differences between groups (−3.6 pmol/L, 95 % CI: −18.0 to 10.8, P = 0.623, Fig. 3A). However, a decrease in ghrelin tAUC was found in the LCD group only (−1142.1 pmol/L*min, 95 % CI: −1850.8 to −433.5, P = 0.002, Fig. 3C), with no change in the low-fat group (−42.9, 95 % CI -842.4 to 756.5, P = 0.912, Fig. 3C). The decrease in tAUC in the LCD was significantly greater compared to the low-fat diet group (−1099.2 pmol/L*min, 95 % CI: −2135.0 to −63.3, P = 0.046, Fig. 3C). No changes over time, or differences between groups, were found for fasting or postprandial plasma concentrations of PYY or GLP-1. Significant decreases in postprandial (both tAUC and iAUC) concentrations of CCK were seen in both the LCD and low-fat diet (−100.1 pmol/L*min, 95 % CI: −148.8 to −51.4, P < 0.001 and −78.6 pmol/L*min, 95 % CI: −133.5 to −23.8, P = 0.005, respectively, Figs. 3N and 2O), with no significant difference between groups.

3.5. Subjective ratings of appetite

No significant between-group differences were observed in subjective ratings of appetite in the fasting or postprandial state (Fig. 4 and Supplementary Table 4). However, only the low-fat diet group reported an increase in subjective ratings of hunger in the fasting state (9.9 mm, 95 % CI: 0.2 to 19.6, P = 0.046, Fig. 4A), while a decrease in postprandial (iAUC) ratings of hunger was seen in both the LCD and low-fat groups (−1968.5 mm*min, 95 % CI: −3323.6 to −613.3, P = 0.004 and −1472.0 mm*min, 95 % CI: −2718.8 to −225.3, P = 0.021, respectively, Fig. 4B). Only the LCD group reported an increase in postprandial (both tAUC and iAUC) ratings of fullness (1723.8 mm*min, 95 % CI: 120.7 to 3326.9, P = 0.035, Fig. 4F and G). Both the LCD and low-fat diet groups reported a decrease in postprandial (tAUC) ratings of PFC (−1248.7 mm*min, 95 % CI: −2017.1 to −480.3, P = 0.001, and −899.1 mm*min, 95 % CI: −1714.5 to −83.6, P = 0.031, respectively, Fig. 4O).

3.5.1. Regression analysis

Changes in fasting ghrelin concentrations were associated with βHB concentrations at w9 (P = 0.013) after adjusting for diet group.

Table 3
Body weight and composition at baseline and week 9, and differences within and between groups.

	Baseline	Week 9	Change from baseline to week 9			Difference in change between groups		
	Mean ± SD	Mean ± SD	EMM	95 % CI	P value	EMM	95 % CI	P value
Body weight, kg								
LCD	103.1 ± 14.0	91.3 ± 12.5	−11.8	−14.1 to −9.5	**<0.001**	−4.3	−7.8 to −0.9	**0.012**
Low-fat diet	102.1 ± 16.7	94.9 ± 17.1	−7.5	−10.0 to −4.9	**<0.001**			
FM, kg								
LCD	50.2 ± 9.6	41.2 ± 8.7	−9.0	−10.6 to −7.4	**<0.001**	−3.6	−5.9 to −1.2	**0.003**
Low-fat diet	50.4 ± 12.5	45.5 ± 12.8	−5.4	−7.2 to −3.7	**<0.001**			
FFM, kg								
LCD	53.0 ± 6.3	49.8 ± 5.5	−2.9	−4.1 to −1.7	**<0.001**	−0.8	−2.5 to 0.9	0.366
Low-fat diet	51.8 ± 6.5	49.4 ± 5.5	−2.1	−3.4 to −0.8	**0.002**			
FM, %								
LCD	48.4 ± 4.3	44.8 ± 4.5	−3.6	−4.5 to −2.8	**<0.001**	−1.7	−3.0 to −0.5	**0.008**
Low-fat diet	48.8 ± 4.9	47.3 ± 5.0	−1.9	−2.9 to −1.0	**<0.001**			

Data presented as mean ± SD. Results from linear mixed model are presented as estimated marginal means (EMM) with corresponding 95 % confidence interval (CI) and p value. N = 30 in LCD and n = 26 in low-fat diet. LCD: low-carbohydrate low-energy diet. EMM: Estimated marginal means. CI: Confidence interval. FM: Fat mass. FFM: Fat free mass.

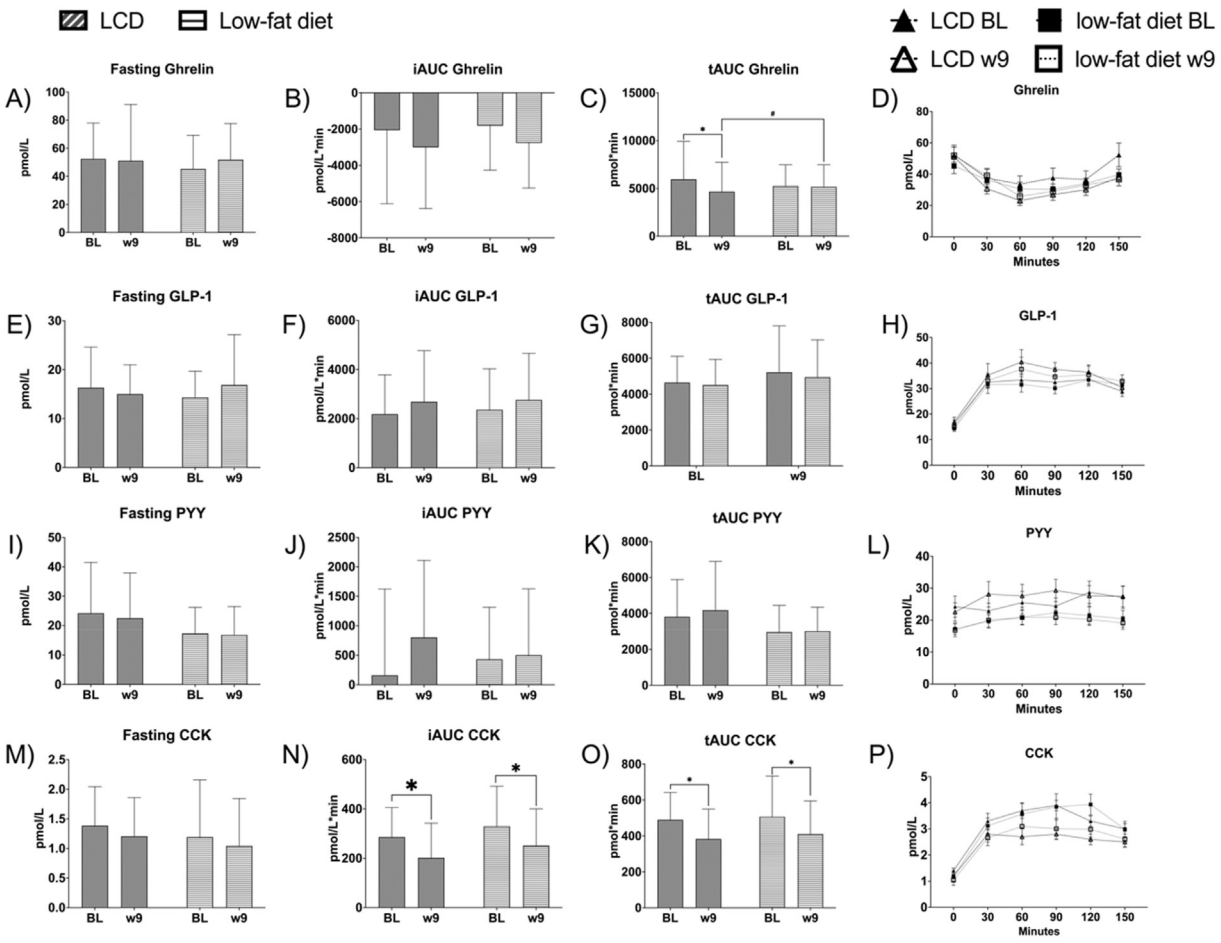

Fig. 3. Fasting and postprandial (tAUC and iAUC) concentrations, and time profile of gastrointestinal hormones, at baseline and after low energy diets, low in carbohydrate (LCD) or fat. A) Fasting acylated ghrelin, B) tAUC and C) iAUC and D) time profile and E) fasting GLP-1, F) GLP-1 iAUC and G) tAUC and H) time profile, I) fasting PYY, J) PYY iAUC and K) tAUC, and L) time profile, M) fasting CCK, N) CCK iAUC and O) tAUC and P) time profile. Data presented as mean ± SD (or mean ± SEM in the time profile). Differences within and between groups were analyzed using linear mixed models. *P < 0.05, significant change from BL to week 9. #P < 0.05, significant difference in change from baseline to week 9 between groups. BL: baseline. w: week. GLP-1: glucagon-like peptide 1. PYY: Peptide YY. CCK: Cholecystokinin.

No other associations were seen for other gastrointestinal hormones or subjective appetite sensations (data not shown).

4. Discussion

The primary objective of this study was to compare changes in fasting ghrelin plasma concentrations after 8-week isocaloric LEDs, either LCD, or low-fat diet, in females with lipedema. Secondary objectives were to determine potential diet group differences in the changes in satiety hormones, as well as subjective appetite ratings. In the present RCT in females with lipedema, fasting plasma ghrelin concentrations did not change following isocaloric LEDs, low in either CHO or fat. However, postprandial ghrelin in response to a standardize meal decreased only on the LCD, and this change was different from the response seen in the low-fat diet group. An increase in subjective ratings of hunger in the fasting state was seen in the low-fat diet group only, and a decrease in postprandial hunger in both groups, while only the LCD group reported an increase in fullness ratings in the postprandial state. Both groups had a decrease in postprandial CCK response.

Contrary to our expectations, there was no increase in ghrelin plasma concentrations in the fasting state in the low-fat group, despite a weight loss of 7.3 % of baseline weight. Most studies report an increase in fasting ghrelin concentrations following diet-induced weight loss, outside of ketosis [12–15]. The lack of increase in fasting ghrelin concentrations in the low-fat diet group, in the present study, may be due to the relatively moderate degree of energy restriction, considering that diets containing ≤1000 kcal/day have not been shown to increase fasting ghrelin concentrations [12,14,15]. Another potential explanation is lack of power. As such, a mean increase in ghrelin concentrations of 4.3 pmol/L (9.5 %) was found in the low-fat group, compared with a mean increase of 0.7 pmol/L (1 %) in the LCD group. This study was powered to investigate the effect of the two diets on pain [8], and as such the present analysis is exploratory. Additionally, fasting concentrations of ghrelin may be affected by sleep [30] and stress [31], which may have increased the within-group variability in ghrelin and masked some of the diet effects.

Fasting plasma concentrations of ghrelin and subjective ratings of hunger remained unaltered on the LCD, as expected in line with previous research [14,15,19,32,33]. No clear threshold for βHB plasma concentrations has been established as a requirement to prevent the increase in ghrelin and hunger otherwise seen with weight loss [34]. However, higher βHB concentrations have been reported to be associated with a greater ghrelin suppression [33], which is also supported by the regression analysis that revealed a negative association between βHB concentration and changes in ghrelin concentrations. Mean βHB plasma concentrations in the LCD group were 0.8 ± 0.4 mmol/L, which based on the present results seems to be sufficient to prevent the increase in subjective

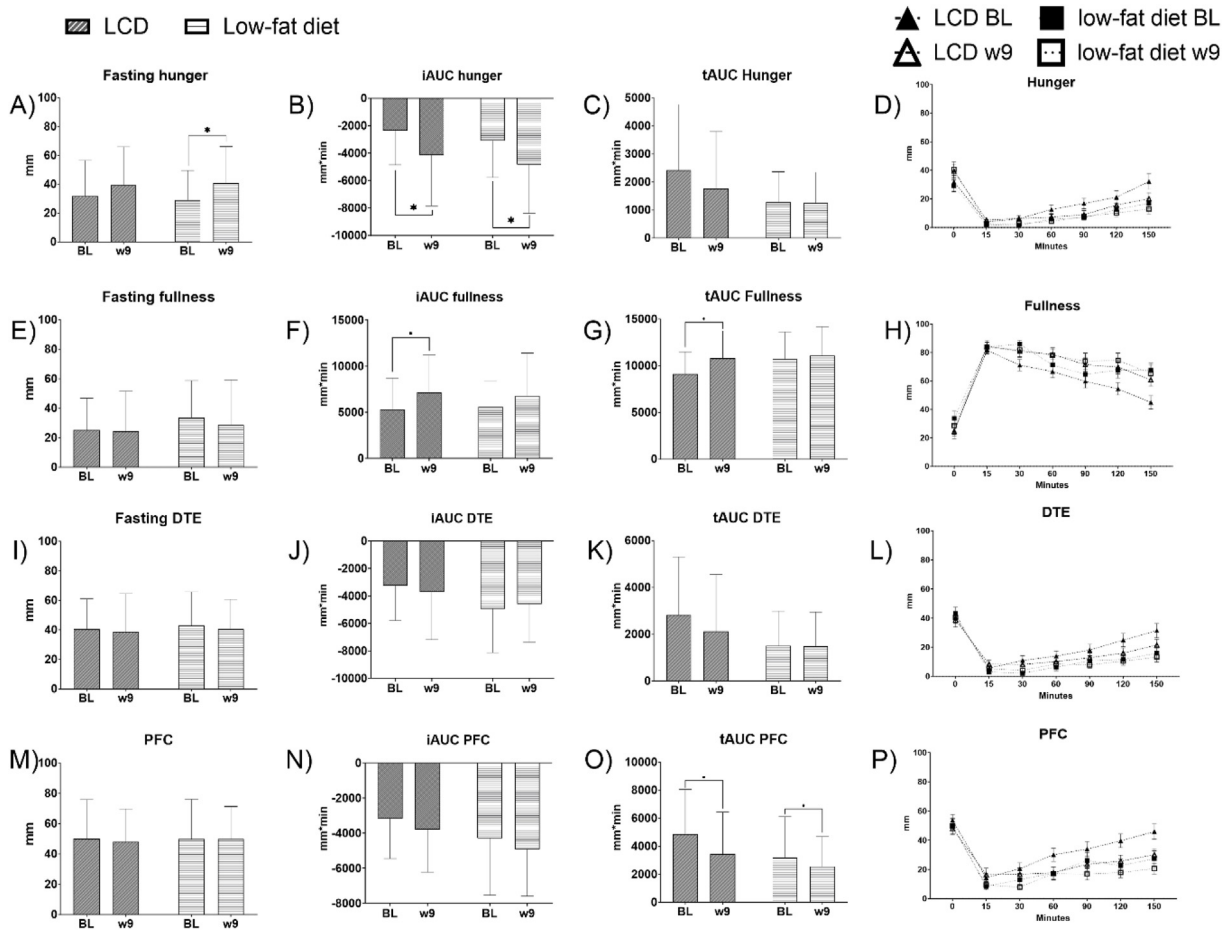

Fig. 4. Subjective ratings of appetite in the fasting and postprandial states and time profile in low-energy diets, low in carbohydrates (LCD) or fat at baseline (BL) and week 9 (W9). A) Hunger in fasting state, B) hunger postprandially iAUC and C) tAUC and D) time profile, A) fullness in fasting state, B) fullness postprandially iAUC and C) tAUC and D) time profile, A) Desire to eat (DTE) in fasting state, B) DTE postprandially iAUC and C) tAUC and D) time profile, A) prospective food consumption (PFC) in fasting state, B) PFC postprandially iAUC and C) tAUC and D) time profile, Data presented as mean ± SD (or mean ± SEM in the time profile). Differences within and between groups were analyzed using linear mixed models. *P < 0.05, significant change from BL to week 9. DTE: Desire to eat. PFC: Prospective food consumption.

ratings of hunger seen in the low-fat diet group. Gibson et al., suggested a threshold of βHB >0.5 mmol/L for subjective hunger ratings to be suppressed [20].

An increase in subjective ratings of postprandial fullness was seen only in the LCD group. This diet group had a higher protein intake (69 g vs. 55 g, P < 0.001), which is known to promote satiety [35]. Our group has previously reported an increase in postprandial fullness after an 8 week VLED [33], while other studies report no change when participants are in ketosis [14,15,19]. However, in the meta-analysis by Gibson et al., the authors concluded that VLEDs lead to an overall increase in feelings of fullness/satiety [20]. Moreover, only the LCD group experienced a reduction in postprandial ghrelin, which might also have contributed to increased postprandial fullness in this group.

Despite an increase in fullness in the LCD group, both groups had a reduction in postprandial CCK response. A decrease in postprandial CCK concentrations has previously been reported in response to weight loss [36]. In the present study, the simultaneous decrease in postprandial CCK, and increase in postprandial ratings of fullness might reflect improved sensitivity to CCK. Diet-induced obesity has been shown to be associated with reduced sensitivity to CCK in animal models [37], which can be expected to improve with weight loss. Probably in part due to changes in hormone sensitivity, subjective appetite ratings and plasma concentrations of GI hormones are often poorly correlated [38]. Postprandial CCK concentrations are usually reduced following energy-restricted diets [18,36], but outcomes when measurements are done under ketogenic conditions remain inconsistent. Some studies report results similar to the present study, with reduction in postprandial CCK [15,19], while others find that CCK concentrations remain unaltered when participants are in ketosis [14,36]. The effect of ketosis on satiety peptides remains unclear and warrants further investigation. Inconsistencies among studies may stem from variations in sample sizes, study design, test meal and sex distribution [19].

No within or between groups differences were found for postprandial concentrations of total GLP-1 and total PYY, which are known to induce satiety. Nymo et al. reported that weight loss induced by a VLED did not affect postprandial concentrations of active GLP-1 and total PYY, while participants were ketotic [15]. On the other hand, Sumithran et al. found a reduction in postprandial total PYY following a VLED, regardless of participants being ketotic or not [14]. The effect of ketosis on satiety hormones remains inconclusive.

The LCD group had a greater loss of body weight compared to the low-fat group, despite no differences between groups in reported energy intake. There was an overall reduction in light physical activity, and an increase in sedentary time over time, however the changes were similar across groups, hence differences in physical activity levels are unlikely to have influenced the results.

It remains unclear whether the greater reduction in body weight and FM observed in the LCD group is a result of better compliance with the diet, not captured in the food diaries, a shift in energy metabolism from CHO to fat oxidation [4], loss of body water due to glycogen depletion [39], or increased energy expenditure [40], despite the latter being controversial.

Despite the prescription of similar protein intake on both diets, the LCD group reported a higher protein intake, as well as a lower fiber intake, compared to the low-fat diet group. This might have affected the results as protein increases the release of the satiety peptides GLP-1 and PYY and is associated with increased satiety [41]. Fiber also has a satiety enhancing effect due to delayed gastric emptying and/or slower absorption of nutrients [35]. However, differences between groups were only significant for ghrelin in the postprandial period, suggesting that differences in protein and fiber intake between groups were unlikely to have affected the results.

Evidence accumulated over the last couple of years shows that low-CHO ketogenic diets are a good treatment option for patients with lipedema, due to their potential in reducing pain [8], and other lipedema symptoms [5–7], while lowering body weight and FM [9]. The present analyses add to previous findings showing that LCDs can suppress appetite during weight loss [20,34,42]. This can improve adherence to energy restricted diets and promote better weight loss outcomes, reinforcing the superiority of LCD in this patient group.

This study has several strengths including its study design and the fact that both plasma concentrations of GI hormones involved in appetite, and subjective appetite ratings, were analyzed. Additionally, the intervention period was 8 weeks ensuring that measurements were taken in the same phase of the menstrual cycle. This is important as phase of menstrual cycle has been shown to modulate appetite [43]. The study also had a low drop-out rate (11 %), and good compliance overall, likely as a result of the tight follow-up. Unfortunate, the study also has limitations. First, the analysis of acylated ghrelin and PYY were done with multiplex kits, which might have lowered the analytic precision. Second, the LCD group reported a higher protein, and a lower fiber intake compared to the low-fat diet group, which might have affected the results. Finally, this is a secondary analysis, and the study may not be sufficiently powered to detect true differences in appetite between the two diets. Therefore, these analyses are exploratory and need to be confirmed in future studies powered specifically for these outcomes.

5. Conclusion

Despite no changes in fasting ghrelin concentrations in either of the diet groups, a reduction in postprandial ghrelin and increased postprandial fullness was seen in the LCD group. These favorable changes in appetite in the LCD group might have contributed to the greater weight loss observed in this group.

Statement of authors' contributions to manuscript

SN, CM and JL designed the study and formulated the research question; JL, MSS and GES carried out the study, JFR and JJH analyzed CCK and GLP-1 concentrations, respectively; JL analyzed the data; SND, RJT, RØ, CM, SN, JL were involved in interpretation of the results; and all authors were involved in writing up the manuscript.

Statement regarding ethics and consent

This study was approved by the Regional Ethics Committee for Medical and Health Research Ethics (REK) (93888). **Informed consent** was obtained from all individual participants included in the study.

Data availability statements

Data described in the manuscript will be available upon request, pending approval the Ethics committee.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2024.11.018.

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