Investigating the effect of sex and ketosis on weight-loss-induced changes in appetite

Anna Lyngstad,[1,2] *Siren Nymo,*[1,3] *Silvia R Coutinho,*[1] *Jens F Rehfeld,*[4] *Helen Truby,*[5] *Bård Kulseng,*[1,2] *and Catia Martins*[1,2]

[1]Obesity Research Group, Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Science, Norwegian University of Science and Technology Trondheim, Norway; [2]Centre for Obesity and Innovation (ObeCe), Clinic of Surgery, St. Olav University Hospital, Trondheim, Norway; [3]Nord-Trøndelag Hospital Trust, Clinic of Surgery, Namsos Hospital, Norway; [4]Department of Clinical Biochemistry, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; and [5]Department of Nutrition, Dietetics & Food, Monash University, Melbourne, Australia

ABSTRACT

Background: Diet-induced weight loss (WL) is usually accompanied by increased appetite, a response that seems to be absent when ketogenic diets are used. It remains unknown if sex modulates the appetite suppressant effect of ketosis.

Objective: The aim of this study was to examine if sex modulates the impact of WL-induced changes in appetite and if ketosis alters these responses.

Methods: Ninety-five individuals (55 females) with obesity (BMI [kg/m ^{2}]: 37 $\pm$ 4) underwent 8 wk of a very-low-energy diet, followed by 4 wk of refeeding and weight stabilization. Body composition, plasma concentration of β-hydroxybutyrate (β-HB) and appetite-related hormones (active ghrelin, active glucagon-like peptide 1 [GLP-1], total peptide YY [PYY], cholecystokinin and insulin), and subjective feelings of appetite were measured at baseline, week 9 in ketosis, and week 13 out of ketosis.

Results: The mean WL at week 9 was 17% for males and 15% for females, which was maintained at week 13. Weight, fat, and fat-free mass loss were greater in males ($P < 0.001$ for all) and the increase in β-HB at week 9 higher in females (1.174 $\pm$ 0.096 compared with 0.783 $\pm$ 0.112 mmol/L, $P = 0.029$). Basal and postprandial GLP-1 and postprandial PYY (all $P < 0.05$) were significantly different for males and females. There were no significant sex $\times$ time interactions for any other appetite-related hormones or subjective feelings of appetite. At week 9, basal GLP-1 was decreased only in males ($P < 0.001$), whereas postprandial GLP-1 was increased only in females ($P < 0.001$). No significant changes in postprandial PYY were observed over time for either sex.

Conclusions: Ketosis appears to have a greater beneficial impact on GLP-1 in females. However, sex does not seem to modulate the changes in the secretion of other appetite-related hormones, or subjective feelings of appetite, seen with WL, regardless of the ketotic state. This trial was registered at clinicaltrials.gov as NCT01834859. *Am J Clin Nutr* 2019;109:1511–1518.

Keywords: ghrelin, glucagon-like peptide 1, peptide YY, cholecystokinin, hunger, fullness, prospective food consumption, ketosis, very-low-energy diet, weight loss

Introduction

Obesity has become a major public health problem worldwide (1). Fortunately, a sustained weight loss (WL) of 5–10% of initial weight is associated with several health benefits, including a reduction in many obesity-related risk factors and comorbidities (2). However, WL is usually followed by an increased drive to eat (3–5). Increased feelings of hunger are thought to be an important contributing factor to the high attrition rate seen in WL attempts and the difficulty in adhering continuously to a dietary energy restriction (6, 7). The compensatory increase in appetite observed during and after WL is thought to be partially driven by changes in the plasma concentration of appetite-related hormones, with an increase in the plasma concentration of the hunger-hormone ghrelin (3, 8), and a reduction in satiety peptides, such as glucagon-like-peptide 1 (GLP-1), peptide YY (PYY), and cholecystokinin (CCK) (3, 9–11).

Interestingly, a review by Gibson et al., in 2015, found that if WL is induced with ketogenic diets, either by a very-low energy diet (VLED) or by a ketogenic low-carbohydrate diet, the drive to eat is absent or reduced while subjects are ketotic (12). This is supported by other studies, which report

This study was funded by the Liaison Committee for education, research, and innovation in Central Norway; the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology.

Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

AL and SN contributed equally.

Address correspondence to SN (e-mail: siren.nymo@ntnu.no).

Abbreviations used: AG, active ghrelin; AUC, area under the curve; CCK, cholecystokinin; FFM, fat-free mass; FM, fat mass; GLP-1, glucagon-like peptide 1; PFC, prospective food consumption; PYY, peptide YY; VLED, very-low-energy diet; WL, weight loss; β-HB, β-hydroxybutyric acid.

Received August 20, 2018. Accepted for publication January 2, 2019.

First published online May 9, 2019; doi: https://doi.org/10.1093/ajcn/nqz002.

no changes in subjective feelings of appetite (13–16), or the plasma concentrations of ghrelin with WL, while participants are ketotic (13, 17–20). However, studies examining the impact of WL, under ketogenic conditions, on the plasma concentration of satiety peptides have mixed results. Three studies reported that plasma concentrations of satiety peptides were unchanged (11, 13, 19), whereas others reported a decrease in active GLP-1, total PYY, and CCK, in both the fasting and postprandial state (15, 17, 18, 21). The majority of the interventions described to date had small sample sizes (11, 13, 16), which may be underpowered to detect changes in all gut peptides. The majority of the studies included in the systematic review and meta-analysis by Gibson et al. were conducted in young females (12). Given that ketosis modulates appetite sensations (22) and the secretion of several appetite-related hormones (23), the question of whether males and females respond differently has not been explored. The aim of this analysis was to assess if sex modulates the changes in objective and subjective measures of appetite associated with WL, both in and out of ketosis.

Study Design, Subjects, and Methods

This paper reports a secondary analysis of the "Weight-loss maintenance and compensatory mechanisms activated with a VLED" study, approved by the Norwegian regional ethics committee (Ref., 2012/1901), registered in clinicaltrials.gov (NCT01834859), and conducted according to the guidelines laid down in the Declaration of Helsinki. All participants provided written informed consent before commencement. The primary outcome of the study was WL maintenance at 1 y, after an initial 8-wk VLED and these results are previously published (24). Secondary outcomes were changes in appetite and energy expenditure in response to the WL induced by the VLED (18, 25). To provide context for this secondary data analysis, subjects were healthy adults (18–65 y, 40 males and 55 females) with obesity ($30 < BMI < 50$ kg/m^2) who were recruited via newspaper advertising serving the community of Trondheim, Norway. Entry criteria were that participants were weight-stable (<2 kg body weight change over the last 3 mo), not currently dieting to lose weight, and with an inactive lifestyle (<150 min of physical activity of at least moderate intensity per week) (26). Owing to the known effect of phase of menstrual cycle on appetite (27), females were postmenopausal, were taking hormonal contraceptives, or had a very regular menstrual cycle (28 ± 2 d). The study excluded pregnant or breastfeeding women and anyone with clinically significant illness, including diabetes, previous WL surgery, and/or taking medication known to affect appetite or induce WL. Participants were provided with an 8-wk ketogenic VLED, followed by 4 wk of refeeding, which was supervised and tailored to individual energy needs by a registered dietician.

Weight-loss phase

Participants followed a ketogenic VLED (Allevo, Karo Pharma AS, Sweden) for 8 wk, 550/660 kcal/d for females and males, respectively (42% carbohydrate, 36% protein, 18% fat, and 4% fiber). No-energy fluids were allowed ad libitum. Intake of low-starch vegetables (max 100 g/d) was encouraged, to provide dietary fiber.

Weight-stabilization phase

At week 9, participants were gradually reintroduced to regular food while reducing the intake of VLED products. An individual diet plan was tailored to individual energy requirements—resting metabolic rate × physical activity level (extracted from individual physical activity monitors [BodyMedia®, SenseWear] at week 8)—with 15–20% protein, 20–30% fat, and 50–60% carbohydrate, which was aimed at weight stabilization (28).

Objective measures of compliance

Diet.

Participants were provided with weekly individual 20-min consultations with a dietician, to review their food records. Urine acetoacetic acid concentration was measured weekly using Ketostix reagent strips (Bayer Corp.). Participants who were not ketotic were educated on how to improve their compliance with the prescribed dietary plan. Participants were considered not compliant and were excluded, if they were not ketotic for more than 1 consecutive week.

Physical activity.

Body Media (SenseWare) armband activity monitors were provided and worn for 7 d at baseline, weeks 8 and 12. Data were considered valid if participants wore the device for ≥4 d, including at least 1 weekend day, on more than 95% (22.8 h/d) of the time (29).

Data collection

The following measurements were performed in the fasting state at baseline, week 9 (the day immediately after the end of the VLED), and week 13.

Body weight and composition.

Air-displacement plethysmography (BodPod, COSMED) was used to record fat mass (FM) and fat-free mass (FFM).

β-Hydroxybutyric acid (β-HB).

The plasma concentration of β-HB in the fasting state was measured with a Ketone Body Assay Kit (Mark134, Sigma-Aldrich).

Appetite measures.

Subjective appetite feelings (hunger, fullness, desire to eat, and prospective food consumption [PFC]) were measured using a validated 10-cm visual analog scale (30), and blood samples were collected in the fasted state and then every 30 min (0, 30, 60, 90, 120, and 150 min) after a standardized breakfast (600 kcal: 17% protein, 35% fat, and 48% carbohydrate) (31, 32), for a period

of 2.5 h. Plasma samples were analyzed for active ghrelin (AG), total PYY, active GLP-1, and insulin using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore) and CCK using an "in-house'' RIA method (33) (intra- and interassay CV were <10% and <20% for AG, GLP-1 and PYY; <10% and <15% for insulin, and <5% and <15% for CCK, respectively).

Power calculation

Sample-size estimation was based on expected differences in appetite suppression while under ketosis, between sexes. For an expected difference of 1 cm in the changes in fasting hunger feelings at week 9 (in ketosis) between sexes, assuming an SD of 1.6 cm, for a power of 80% and a statistical significance of 0.05, 84 participants would be needed. To allow for an expected drop-out rate of 15%, 97 participants were deemed necessary.

Statistical analysis

Statistical analysis was performed using SPSS version 24 (SPSS Inc.), and data presented as estimated marginal mean ± SEM, except for baseline characteristics where mean ± SD are given. Statistical significance was set at $P < 0.05$. Data were analyzed using linear mixed-effects models, with restricted maximum-likelihood estimation, including fixed effects for time and sex, and their interaction. The Benjamini–Hochberg method was used for main effects and post hoc pairwise comparisons, which controls for the false discovery rate, to adjust for the fact that we have examined a large number of variables (34). Results are reported for all completing participants if no significant sex × time interaction was found and separately for males and females if a significant sex × time interaction was present.

The total AUC for subjective feelings of appetite and appetite hormones was calculated from 0 to 150 min using the trapezoid rule. Correlations between β-HB plasma concentrations and changes in appetite were analyzed using the Spearman rank correlation test. All participants with available data on at least 2 out of the 3 time-points were considered completers and included in the analysis.

Results

One hundred subjects met the study entry criteria and started the study. Five males did not complete the 8-wk VLED (2 did not tolerate the VLED, 1 was excluded owing to consumption of extra food, 1 withdrew for personal reasons, and 1 was lost to follow-up), and 1 female did not complete measurements at week 13 (withdrew due to family illness). Ninety-five participants (55 females; 15 postmenopausal, 9 with a very regular menstrual cycle, and 21 taking hormonal contraceptives) were, therefore, included in this completer's analysis with a mean age of 43 ± 10 y, and a BMI of 37 ± 4. Males were significantly younger (40 ± 9 compared with 44 ± 10 y, for males and females respectively, $P < 0.01$) and had a higher body weight at baseline than females (119.8 ± 19 compared with 102.3 ± 12.8 kg, $P < 0.001$, respectively), but there were no differences in BMI.

Compliance

Diet.

Compliance with the VLED was excellent, and no participant was excluded based on negative acetoacetic acid concentration in the urine.

Physical activity.

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

Body weight and body composition

Changes in body weight and body composition are reported in **Table 1**. Eight weeks of following the VLED resulted in a 15.9% (17.9 ± 0.4 kg) WL, which was sustained at week 13 (18.4 ± 0.5 kg). A significant sex × time interaction was observed for weight, FM, and FFM ($P < 0.001$ for all). Males had a greater weight (kg and %), FM, and FFM (kg) loss than females at week 9 (17% [20.6 ± 0.5 kg] compared with 15% (15.3 ± 0.5 kg), $P < 0.001$ for both, for body weight, 15.5 ± 0.5 compared with 12.2 ± 0.4 kg, $P < 0.001$ for FM and 4.9 ± 0.3 compared with 3.1 ± 0.3 kg, $P < 0.01$ for FFM, respectively). These differences were also apparent at week 13, with males achieving a greater reduction in weight, FM, and FFM (from baseline) than females.

Ketosis

Changes in β-HB plasma concentration over time are reported in Table 1. β-HB increased significantly between baseline and week 9 ($P < 0.001$ for all) and decreased between week 9 and 13 ($P < 0.001$ for all), with concentration at week 13 no longer being different from baseline. A significant sex × time interaction was present ($P = 0.029$), with a smaller increase in β-HB plasma concentration, from baseline to week 9, in males than in females (0.783 ± 0.112 compared with 1.174 ± 0.096 mmol/L, respectively).

Appetite-related hormones

Changes in basal plasma concentration of appetite-related hormones are reported in **Table 2**. There were no significant sex × time interactions for basal plasma concentration of appetite-related hormones, with the exception of active GLP-1 ($P = 0.048$). No significant changes from baseline were seen for basal AG concentration at week 9, under ketosis, in all participants. Basal AG plasma concentrations increased significantly from week 9 to 13 ($P < 0.001$) and were significantly higher than baseline at week 13 ($P < 0.001$). A significant decrease in basal GLP-1 plasma concentration was clear at week 9 in males only ($P < 0.001$). No changes over time occurred for basal total PYY plasma concentration. Basal plasma concentrations of CCK decreased significantly from baseline to week 9 ($P < 0.001$) and

TABLE 1 Baseline and changes in body weight and composition, and β-hydroxybutyric acid plasma concentrations over time in males ($n = 40$) and females ($n = 55$)[1]

	Baseline	Δ Baseline–week 9	Δ Baseline–week 13	Δ Week 9–week 13	P value for sex × time interaction
Body weight, kg					
Males	119.8 ± 2.3	−20.6 ± 0.5***	−20.3 ± 0.6***	0.2 ± 0.6	<0.001
Females	102.3 ± 1.9	−15.3 ± 0.5**	−16.5 ± 0.8**	−1.1 ± 08	
Fat mass, kg					
Males	47.0 ± 1.6	−15.5 ± 0.5***	−18.0 ± 0.6***	−2.5 ± 0.6***	<0.001
Females	49.2 ± 1.4	−12.2 ± 0.4***	−14.4 ± 0.8***	−2.2 ± 0.8**	
Fat-free mass, kg					
Males	71.6 ± 1.0	−4.9 ± 0.3***	−3.2 ± 0.3	1.7 ± 0.3***	<0.001
Females	53.0 ± 0.9	−3.1 ± 0.3***	−1.8 ± 0.4	1.3 ± 0.4**	
β-Hydroxybutyric acid, mmol/L					
Males	0.128 ± 0.078	0.783 ± 0.112***	0.009 ± 0.112	−0.774 ± 0.112***	0.029
Females	0.152 ± 0.069	1.174 ± 0.096***	−0.004 ± 0.153	−1.176 ± 0.153***	

[1] Values are estimated marginal means ± SEMs. Data were analyzed using linear mixed-effects models with restricted maximum-likelihood estimation, including fixed effects for time, sex, and their interaction. Symbols denote significant changes overtime: ***$P < 0.001$, **$P < 0.01$. Males had a significantly larger weight, fat mass, and fat-free mass loss ($P < 0.001$ for all) and a smaller increase in β-hydroxybutyric acid at week 9 ($P = 0.029$) than females.

then increased with refeeding ($P < 0.001$), with concentrations at week 13 being similar to baseline values. Basal insulin plasma concentration was significantly decreased at both week 9 and week 13, compared with baseline ($P < 0.001$ for both). Postprandial plasma concentrations of appetite hormones are listed in Table 2.

Supplemental Figure 1 provides a graphical version of the AUC. There was no significant sex × time interaction for

TABLE 2 Baseline and changes in basal plasma concentrations and AUC of appetite-related hormones over time in all participants ($n = 95$), males ($n = 40$) and females ($n = 55$)[1]

	Baseline	Δ Baseline–week 9	Δ Baseline–week 13	Δ Week 9–week 13	P value for sex × time interaction
Basal					
Active ghrelin, pg/mL	—	—	—	—	0.211
All	96.9 ± 8.1	3.6 ± 6.9	43.4 ± 9.0***	39.8 ± 9.0***	
Active glucagon-like peptide 1, pg/mL	_	_	_	_	0.048
Males	9.4 ± 2.8	−5.0 ± 1.2***	−1.7 ± 1.3	3.3 ± 1.3	
Females	6.4 ± 2.4	−0.8 ± 1.1	−2.0 ± 2.0	−1.2 ± 2.0	
Total peptide YY, pg/mL	—	—	—	—	0.497
All	47.0 ± 6.0	−12.1 ± 5.1	0.7 ± 6.6	12.8 ± 6.6	
Cholecystokinin, pmol/L	—	—	—	—	0.593
All	1.02 ± 0.06	−0.26 ± 0.07***	0.07 ± 0.09	0.33 ± 0.09***	
Insulin, pg/mL	—	—	—	—	0.162
All	1098 ± 53	−715 ± 57***	−604 ± 74***	111 ± 74	
Postprandial, AUC					
Active ghrelin, pg/mL·min	—	—	—	—	0.141
All	10,383 ± 835	662 ± 599	4559 ± 777***	−3897 ± 777***	
Active glucagon-like peptide 1, pg/mL·min	_	_	_	_	0.015
Males	2010 ± 365	−171 ± 180	−349 ± 194	−179 ± 194	
Females	1939 ± 322	573 ± 162***	−316 ± 290	−889 ± 290**	
Total peptide YY, pg/mL·min	—	—	—	—	0.016
Males	9803 ± 1354	−1317 ± 1040	−1232 ± 1151	85 ± 1151	
Females	7683 ± 1233	3077 ± 970	−798 ± 1577	−3875 ± 1579	
Cholecystokinin, pmol/L·min	—	—	—	—	0.723
All	388 ± 15	−100 ± 12***	−26 ± 15	74 ± 15***	
Insulin, pg/mL·min	—	—	—	—	0.262
All	626,864 ± 31,193	−265,956 ± 28,440***	−287,575 ± 36,879***	−21,619 ± 36,903	

[1] Values are estimated marginal means ± SEMs. Data were analyzed using linear mixed-effects models with restricted maximum-likelihood estimation, including fixed effects for time, sex, and their interaction. Symbols denote significant changes overtime: ***$P < 0.001$, **$P < 0.01$. Only males experienced a significant reduction in basal active glucagon-like peptide 1 from baseline to week 9. Only females experienced an increase in active glucagon-like peptide 1 AUC from baseline to week 9. AUC, area under the curve.

TABLE 3 Baseline and changes in subjective feelings of appetite during fasting and AUC over time in all participants ($n = 95$)[1]

	Baseline	Δ Baseline–week 9	Δ Baseline–week 13	Δ Week 9–week 13	P value for sex × time interaction
Fasting					
Hunger, cm	—	—	—	—	0.43
All	3.8 ± 0,2	0.6 ± 0.3*	1.5 ± 0.4***	0.9 ± 0.4*	
Fullness, cm	—	—	—	—	0.581
All	2.3 ± 0.2	0.5 ± 0.2	0.4 ± 0.3	− 0.2 ± 0.3	
Desire to eat, cm	—	—	—	—	0.928
All	4.5 ± 0.2	0.01 ± 0.2	0.8 ± 0.3*	0.8 ± 0.3*	
Prospective food consumption, cm	—	—	—	—	0.468
All	5.9 ± 0.2	− 1.1 ± 0.3***	− 0.3 ± 0.3	0.8 ± 0.3*	
Postprandial (AUC)					
Hunger, cm·min	—	—	—	—	0.309
All	342.0 ± 23.7	− 49.8 ± 22.0**	22.0 ± 28.6	71.8 ± 28.6*	
Fullness, cm·min	—	—	—	—	0.134
All	876.1 ± 26.0	209.8 ± 30.4***	84.3 ± 38.8	− 125.6 ± 38.9**	
Desire to eat, cm·min	—	—	—	—	0.184
All	415.0 ± 27.6	− 66.7 ± 23.9*	− 16.2 ± 31.1	50.5 ± 31.1	
Prospective food consumption, cm·min	—	—	—	—	0.843
All	659.5 ± 32.8	− 184.9 ± 32.2***	− 162.4 ± 41.7***	22.5 ± 41.7	

[1]Values are estimated marginal means ± SEMs. Data were analyzed using linear mixed-effects models with restricted maximum-likelihood estimation, including fixed effects for time, sex, and their interaction. Symbols denote significant changes overtime: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. The changes in subjective appetite feelings over time were not statistically significant different between sexes. AUC, area under the curve.

postprandial plasma concentration of appetite-related hormones, with the exception of active GLP-1 ($P = 0.015$) and PYY ($P = 0.016$). No significant change in the AUC for AG was recorded between baseline and week 9, but by week 13 when refeeding occurred, there was a significant increase from baseline and from week 9 ($P < 0.001$ for both). A significant increase in active GLP-1 AUC was seen in females only at week 9 ($P < 0.001$), followed by a significant fall between weeks 9 and 13 ($P < 0.0001$). No significant changes were found for active GLP-1 AUC at week 13, compared with baseline, in either males or females. Despite a significant sex × time interaction for total PYY AUC, no significant sex differences over time were apparent.

A significant reduction in CCK AUC was clearly seen while participants were ketotic at week 9 ($P < 0.001$), and an increase from week 9 to week 13 ($P < 0.001$), with CCK AUC at week 13 being no longer different from baseline. A significant reduction in insulin AUC was observed with WL at both weeks 9 and 13, compared with baseline ($P < 0.001$ for both).

Subjective feelings of appetite

Changes in subjective appetite feelings in the fasting state are listed in **Table 3**. No significant sex × time interaction was apparent for subjective feelings of appetite in the fasting state. A significant increase in hunger was clear at week 9, when participants were ketotic ($P < 0.05$), followed by a further rise with refeeding ($P < 0.05$). No significant changes overtime were found for fullness. A significant increase was seen in feelings of desire to eat at week 13 ($P < 0.05$), but not at week 9, compared with baseline. A significant decrease in feelings of PFC was reported at week 9 ($P < 0.001$), but not at week 13, compared with baseline.

Postprandial ratings of appetite over time are listed in Table 3. **Supplemental Figure 2** displays the AUC observations graphically. No significant sex × time interaction was recorded for postprandial ratings of appetite. Hunger AUC decreased from baseline to week 9 ($P < 0.01$), followed by an increase with refeeding (week 13: $P < 0.05$). No significant differences were seen in hunger AUC between baseline and week 13. Participants rated their feelings of fullness as increased from baseline to week 9 (AUC, fullness $P < 0.001$), which was followed by a decrease with refeeding, by week 13 ($P < 0.01$). These subjective feelings of fullness returned to baseline levels, with no significant change in AUC fullness between baseline and week 13.

Participants rated their desire to eat similarly, with a significant reduction in desire to eat at week 9 ($P < 0.05$) and returning to baseline levels by week 13. The AUC for PFC also decreased significantly at week 9 ($P < 0.001$) and remained lower than baseline at week 13 ($P < 0.001$).

Correlation analysis

A higher β-HB concentration at week 9 was associated with a greater increase in active GLP-1 AUC (from baseline to week 9) in females only ($\rho = 0.4$, $P < 0.01$). A greater WL at week 13 was associated with a greater increase in hunger ($\rho = 0.6$, $P < 0.05$) and desire to eat ($\rho = 0.71$, $P < 0.01$) feelings in the fasted state. There were no significant correlations between either β-HB plasma concentration or WL (kg/%) and other appetite markers.

Discussion

The aim of this analysis was to investigate if sex modulates the changes in appetite seen with WL, both in and out of ketosis. This

was achieved by secondary analysis of a longitudinal weight-loss study where healthy adult participants undertook a VLED diet for 8 wk to induce ketosis and then transitioned to a food-based weight maintenance diet and reversal of the ketotic state between week 9 and week 13 (19). With the exception of changes in basal and postprandial GLP-1, which were more favorable in females while in ketosis, the changes in both plasma concentration of appetite-related hormones and subjective feelings of appetite seen with WL, both in and out of ketosis, were similar for both males and females.

Basal and postprandial AG plasma concentrations were unaltered while participants were ketotic, despite a 16% WL. These finding corroborate with previous evidence showing that WL induced by ketogenic diets has no impact on active (17–20) or total ghrelin (13). Furthermore, an increase in AG was seen after refeeding and outside ketosis, which concurs with the majority of the literature (3, 8, 17, 18, 35). However, Moran et al. in 2007 reported no changes in basal or AUC for total ghrelin after a 4.2-kg WL induced by 8 wk of an energy-restricted diet, in females (36), and others have reported no changes in basal AG concentration after WL in mixed samples of males and females (37, 38). The WL in these studies was nevertheless modest (4–7 kg), which suggests that the increase in AG, seen outside ketosis, is only apparent after a certain threshold of WL or body fat loss. This is supported by others, which found an increase in ghrelin plasma concentrations with a WL >10% (3, 18, 35).

Some sex differences were apparent for active GLP-1. During ketosis, only males had a significant decrease in basal GLP-1, whereas females had a significant increase in active GLP-1 AUC. The evidence regarding the impact of WL under ketosis on GLP-1 secretion is, however, inconsistent, with unchanged basal and postprandial active GLP-1 in females (19), decreased basal and unchanged AUC active GLP-1 (17), and a decrease in AUC active GLP-1 in mixed samples (15) being described. No changes in active GLP-1 concentrations were recorded after refeeding when participants were not ketotic, which is similar to previous reports, for both basal (19, 35, 39, 40) and in the postprandial period for GLP-1 (3, 19, 39). Interestingly, both Verdich et al. (males only) (40) and Iepsen et al. (primarily females) (35) reported an increase in total GLP-1 AUC with WL when participants were not ketotic, following a low-calorie diet (WL: 18.8 kg and 12.5 kg, respectively). Thus, both the fraction of the hormone and the methods of measurement could explain the divergent results for GLP-1 (41) and need to be considered when comparing changes in GLP-1 concentrations after WL. Different hormone fractions could also explain divergent results for PYY. Both an increase in postprandial $PYY_{3–36}$ (35) and a decrease in mean values of total PYY (3) have been reported after WL in mixed samples. Our study did not reveal any significant changes in total PYY plasma concentration either in or out of ketosis, which assists in substantiating the available evidence to date (13, 18, 36).

Unexpectedly, basal and postprandial CCK concentrations fell during ketosis, whereas no changes from baseline were seen after 4 wk of refeeding. Chearskul et al. in 2008 reported no changes in postprandial CCK in males when subjects were in ketosis, but after 1 wk of refeeding, postprandial CCK was decreased compared with both baseline and during ketosis (11). Others have observed a decrease in basal CCK in ketosis, whereas both basal and postprandial CCK were decreased after 2 wk of refeeding in a mixed sample (17). On the other hand, an increase in basal and postprandial CCK was reported after a 4.5-kg WL in another study (37). Thus, the available evidence regarding how CCK plasma concentrations change with WL, in or out of ketosis, remains inconsistent. Different study designs, sample sizes, sex distribution, assay specificity, and length of refeeding period may explain the divergent results.

Even though an increase in hunger feelings when fasted was apparent with WL and during ketosis, it needs to be highlighted that this increase was less than half that seen with the same WL out of ketosis. This strengthens the evidence that ketosis has a suppressant effect on appetite. The increase in postprandial fullness, and reduction in postprandial desire to eat, seen with WL during ketosis is in agreement with Ratliff et al. who reported in 2009 the effects of a ketogenic low-carbohydrate diet on appetite in males (13). Contrary to our findings, no changes in subjective feelings of appetite while in the fasting state or after a meal during ketosis were reported in other studies (11, 17, 18). However, our analysis has the largest sample size to date, so it may be very possible that previous studies were underpowered to detect subtle changes in appetite.

When out of ketosis (week 13), an increase in hunger was observed in the fasting state, which concurs with the majority of the literature (17, 18, 20, 42). After a meal, feelings of PFC were reduced. It remains uncertain if 4 wk of refeeding and weight stabilization is sufficient for energy balance to be re-established in subjects with obesity.

Interestingly, our findings show little correlation between β-HB plasma concentration and changes in hunger or AG during ketosis. The only correlation observed was for a higher ketosis to be associated with a larger GLP-1 AUC in females. Even though the appetite suppressant effects of ketogenic diets is well established (12), the exact mechanisms mediating specifically the absence (or lower) increase in hunger and ghrelin while in ketosis remain to be fully elucidated (43).

The present analysis has several strengths. First, the sample size is much larger than in previous studies and has a more balanced sex distribution. Second, both objective and subjective appetite markers during fasting plus in response to a meal were assessed. Third, compliance with the diet was excellent, and participants remained weight-stable after refeeding, with objective measures of compliance by using both urinary and blood ketone levels at all time-points. This analysis also has some limitations. A multiplex kit was used to measure AG, active GLP-1, total PYY, and insulin, which is likely to result in less accurate and precise measurements than optimized assays for each individual hormone. Moreover, the energy load of the standardized meal was not adjusted for body size, so that the larger active GLP-1 secretion reported in the postprandial state in females only could potentially be due to the fact that the test meal represented a larger proportion of energy needs in females compared with males. As the mean age difference was 4 y, we have not adjusted for age in our analysis, and even though some research suggests that subjective feelings of appetite may change with aging (22), this difference was unlikely to have any substantial impact. Finally, the intake of low-starch vegetables during the ketotic phase of the diet was not quantified or taken into account in the analysis, as we relied on objective measures of ketosis as a measure of diet compliance.

Ketogenic VLEDs induce WL effectively in adults, both males and females, and should, therefore, be considered as a potential

option for WL treatment. Both clinicians and patients with obesity should be aware that both males and females are likely to experience an increase in hunger in the fasting state, despite being ketotic (even though less than half of what would be expected for the same WL when not ketotic), and that a further increase in the drive to eat should be expected after refeeding. Participant-centered strategies need to be in place to reduce the risk of overeating and relapse in the long-term to help prevent weight regain.

In conclusion, even though ketosis seems to have a more beneficial impact on GLP-1 secretion in females, sex alone does not appear to modulate the secretion of gut peptides that signal hunger and satiety. Increased subjective feelings of hunger with WL should be anticipated in adults regardless of the ketotic state. Ketosis can minimize the expected increase in hunger apparent after WL in both males and females.

We would like to thank Hege Bjøru and Sissel Salater (at the ObeCe, Clinic of Surgery, St. Olav University Hospital) for support with screening and blood collection, Turid Follestad for help with statistical analysis, and Ingrid Hals for support with laboratory work (both at the Department of Clinical and Molecular Medicine, NTNU).

The authors' responsibilities were as follows—CM, HT, and BK: designed the research; SN and AL: conducted the research, analyzed the data, and wrote the paper; JFR: performed the analysis of CCK; CM: had primary responsibility for the final content; and all authors: read and approved the final manuscript. None of the authors report a conflict of interest related to research presented in this article.

References

1. WHO. Obesity and overweight. [Internet]. 2015 [cited 2018 May 2]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/.
2. Ryan DH, Yockey SR. Weight loss and improvement in comorbidity: Differences at 5%, 10%, 15%, and over. Curr Obes Rep 2017;6(2):187–94.
3. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med 2011;365(17):1597–604.
4. Gilbert JA, Drapeau V, Astrup A, Tremblay A. Relationship between diet-induced changes in body fat and appetite sensations in women. Appetite 2009;52(3):809–12.
5. Doucet E, St-Pierre S, Almeras N, Tremblay A. Relation between appetite ratings before and after a standard meal and estimates of daily energy intake in obese and reduced obese individuals. Appetite 2003;40(2):137–43.
6. Polidori D, Sanghvi A, Seeley RJ, Hall KD. How strongly does appetite counter weight loss? Quantification of the feedback control of human energy intake. Obesity (Silver Spring, Md) 2016;24(11):2289–95.
7. Pasman WJ, Saris WH, Westerterp-Plantenga MS. Predictors of weight maintenance. Obes Res 1999;7(1):43–50.
8. Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 2002;346(21):1623–30.
9. Essah PA, Levy JR, Sistrun SN, Kelly SM, Nestler JE. Effect of weight loss by a low-fat diet and a low-carbohydrate diet on peptide YY levels. Int J Obes (Lond) 2010;34(8):1239–42.
10. de Luis DA, Gonzalez Sagrado M, Conde R, Aller R, Izaola O. Decreased basal levels of glucagon-like peptide-1 after weight loss in obese subjects. Ann Nutr Metab 2007;51(2):134–8.
11. Chearskul S, Delbridge E, Shulkes A, Proietto J, Kriketos A. Effect of weight loss and ketosis on postprandial cholecystokinin and free fatty acid concentrations. Am J Clin Nutr 2008;87(5):1238–46.
12. Gibson AA, Seimon RV, Lee CM, Ayre J, Franklin J, Markovic TP, Caterson ID, Sainsbury A. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. Obes Rev 2015;16(1):64–76.
13. Ratliff J, Mutungi G, Puglisi MJ, Volek JS, Fernandez ML. Carbohydrate restriction (with or without additional dietary cholesterol provided by eggs) reduces insulin resistance and plasma leptin without modifying appetite hormones in adult men. Nutr Res 2009;29(4):262–8.
14. Hursel R, Westerterp-Plantenga MS. Green tea catechin plus caffeine supplementation to a high-protein diet has no additional effect on body weight maintenance after weight loss. Am J Clin Nutr 2009;89(3):822–30.
15. Adam TC, Jocken J, Westerterp-Plantenga MS. Decreased glucagon-like peptide 1 release after weight loss in overweight/obese subjects. Obes Res 2005;13(4):710–6.
16. Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. Am J Clin Nutr 2008;87(1):44–55.
17. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Ketosis and appetite-mediating nutrients and hormones after weight loss. Eur J Clin Nutr 2013;67(7):759–64.
18. Nymo S, Coutinho SR, Jorgensen J, Rehfeld JF, Truby H, Kulseng B, Martins C. Timeline of changes in appetite during weight loss with a ketogenic diet. Int J Obes (Lond) 2017;41(8):1224–31.
19. Diepvens K, Soenen S, Steijns J, Arnold M, Westerterp-Plantenga M. Long-term effects of consumption of a novel fat emulsion in relation to body-weight management. Int J Obes (Lond) 2007;31(6):942–9.
20. Coutinho SR, With E, Rehfeld JF, Kulseng B, Truby H, Martins C. The impact of rate of weight loss on body composition and compensatory mechanisms during weight reduction: A randomized control trial. Clin Nutr 2018;37(4):1154–62.
21. Coutinho SR, Halset EH, Gasbakk S, Rehfeld JF, Kulseng B, Truby H, Martins C. Compensatory mechanisms activated with intermittent energy restriction: A randomized control trial. Clin Nutr 2018;37(3):815–23.
22. Gregersen NT, Moller BK, Raben A, Kristensen ST, Holm L, Flint A, Astrup A. Determinants of appetite ratings: The role of age, gender, BMI, physical activity, smoking habits, and diet/weight concern. Food Nutr Res 2011;55.
23. Asarian L, Geary N. Sex differences in the physiology of eating. Am J Physiol Regul Integr Comp Physiol 2013;305(11):R1215–67.
24. Nymo S, Coutinho SR, Eknes PH, Vestbostad I, Rehfeld JF, Truby H, Kulseng B, Martins C. Investigation of the long-term sustainability of changes in appetite after weight loss. Int J Obes (Lond) 2018;42(8):1489–99.
25. Nymo S, Coutinho SR, Torgersen LH, Bomo OJ, Haugvaldstad I, Truby H, Kulseng B, Martins C. Timeline of changes in adaptive physiological responses, at the level of energy expenditure, with progressive weight loss. Br J Nutr 2018;120(2):141–9.
26. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, et al. Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007;39(8):1423–34.
27. Brennan IM, Feltrin KL, Nair NS, Hausken T, Little TJ, Gentilcore D, Wishart JM, Jones KL, Horowitz M, et al. Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. Am J Physiol Gastrointest Liver Physiol 2009;297(3):G602–10.
28. Nordic Nutrition Recommendations 2012 (NNR2012) Copenhagen: norden.org; 2014 [Internet]. [cited 2018 May 3]. Available from: https://www.norden.org/en/theme/former-themes/themes-2016/nordic-nutrition-recommendation/nordic-nutrition-recommendations-2012.
29. Scheers T, Philippaerts R, Lefevre J. Patterns of physical activity and sedentary behavior in normal-weight, overweight and obese adults, as measured with a portable armband device and an electronic diary. Clin Nutr 2012;31(5):756–64.
30. Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, Stratton R, Delargy H, King N, et al. The use of visual analogue scales to assess motivation to eat in human subjects: A review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Br J Nutr 2000;84(4):405–15.

31. Martins C, Kulseng B, King NA, Holst JJ, Blundell JE. The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. J Clin Endocrinol Metab 2010;95(4): 1609–16.
32. Martins C, Aschehoug I, Ludviksen M, Holst J, Finlayson G, Wisloff U, Morgan L, King N, Kulseng B. High-intensity interval training, appetite, and reward value of food in the obese. Med Sci Sports Exerc 2017;49(9):1851–8.
33. Rehfeld JF. Accurate measurement of cholecystokinin in plasma. Clin Chem 1998;44(5):991–1001.
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Statist Soc Ser A 1995;Series B(57):289–300.
35. Iepsen EW, Lundgren J, Holst JJ, Madsbad S, Torekov SS. Successful weight loss maintenance includes long-term increased meal responses of GLP-1 and PYY3-36. Eur J Endocrinol 2016;174(6): 775–84.
36. Moran LJ, Noakes M, Clifton PM, Wittert GA, Le Roux CW, Ghatei MA, Bloom SR, Norman RJ. Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome. Am J Clin Nutr 2007;86(6):1603–10.
37. Beck EJ, Tapsell LC, Batterham MJ, Tosh SM, Huang XF. Oat beta-glucan supplementation does not enhance the effectiveness of an energy-restricted diet in overweight women. Br J Nutr 2010;103(8):1212–22.
38. de Luis DA, Sagrado MG, Conde R, Aller R, Izaola O. Changes of ghrelin and leptin in response to hypocaloric diet in obese patients. Nutrition 2008;24(2):162–6.
39. Adam TC, Lejeune MP, Westerterp-Plantenga MS. Nutrient-stimulated glucagon-like peptide 1 release after body-weight loss and weight maintenance in human subjects. Br J Nutr 2006;95(1): 160–7.
40. Verdich C, Toubro S, Buemann B, Lysgard Madsen J, Juul Holst J, Astrup A. The role of postprandial releases of insulin and incretin hormones in meal-induced satiety–effect of obesity and weight reduction. Int J Obes Relat Metab Disord 2001;25(8): 1206–14.
41. Heijboer AC, Frans A, Lomecky M, Blankenstein MA. Analysis of glucagon-like peptide 1; what to measure? Clin Chim Acta 2011;412(13-14):1191–4.
42. Diepvens K, Kovacs EM, Nijs IM, Vogels N, Westerterp-Plantenga MS. Effect of green tea on resting energy expenditure and substrate oxidation during weight loss in overweight females. Br J Nutr 2005;94(6): 1026–34.
43. Paoli A, Bosco G, Camporesi EM, Mangar D. Ketosis, ketogenic diet and food intake control: A complex relationship. Front Psychol 2015;6:27.