Differences in gastrointestinal hormones and appetite ratings among obesity classes

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ABSTRACT

The aim of this study was to compare gastrointestinal (GI) hormones and subjective ratings of appetite among obesity classes, and between classes of obesity and controls. Ninety-eight adult individuals with obesity, divided into class I (n = 35), II (n = 44) and III (n = 19), together with 45 controls without obesity were included in this cross-sectional analysis. Body weight/composition, and basal and postprandial (after a 600 kcal fixed breakfast) plasma concentrations of acylated ghrelin, active glucagon-like peptide 1 (GLP-1), total peptide YY (PYY), cholecystokinin (CCK) and insulin, as well as subjective ratings of hunger, fullness, desire to eat (DTE) and prospective food consumption (PFC) were measured. There were no differences in the plasma concentration of GI hormones (either basal or postprandial) among obesity classes, except for insulin. In general, obesity was associated with impaired secretion of GI hormones. Ghrelin secretion did not decline postprandially in class-III obesity. GLP-1 peak for obesity class I and II was early and lower, while class III showed no postprandial GLP-1 response. Postprandial PYY response for obesity class II and III was absent, and class III showed a delayed and shortened postprandial CCK response. Obesity class II and III had greater basal insulin concentration compared to controls and postprandial insulin was greater in obesity class III versus class II, class I and controls. No differences were found for appetite ratings among obesity classes. In conclusion, obesity is characterized by impaired secretion of GI hormones, with reduced postprandial satiety, particularly in individuals with obesity class III. This abnormal pattern may lead to overeating.

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1. Introduction

Obesity is a chronic, progressive, and relapsing disease (Bray, Kim, & Wilding, 2017) classified by body mass index (BMI) ≥30 kg/m². It is further divided into subclasses according to the degree of severity: class I (BMI: 30.0–34.9 kg/m²), class II (BMI: 35.0–39.9 kg/m²), and class III (BMI: ≥40.0 kg/m²), with the latter two defined as severe obesity (WHO, 2000).

Obesity results from a chronic positive energy imbalance (Hall & Guo, 2017), and the rising prevalence is most likely driven by increased accessibility to highly palatable foods and increased portion sizes (Westerterp & Speakman, 2008). Adverse health outcomes increase with BMI, and a BMI ≥40.0 kg/m² is associated with approximately 6–14 years shortened life expectancy (Walls et al., 2010). It is therefore concerning that the greatest increases in prevalence have been seen in the classes of severe obesity (Walls et al., 2010).

In normal conditions, the appetite control system senses both acute and chronic changes in nutritional status, and gastrointestinal (GI) hormones have been hypothesized to act as physiological signals. Food ingestion is followed by the suppression of ghrelin, thought to signal...
hunger, and the secretion of glucagon-like peptide 1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK) involved in both meal-termination (or satiation) and inter-meal interval (or satiety) (Steinert et al., 2017). Individuals with obesity seem to have a dysregulated appetite control. We (DeBenedictis et al., 2020) and others (Daghestani, Ozand, Aylwin, et al., 2006; Mans, Serra-Prat, Palomera, Sunol, & Clave, 2015) have previously shown that individuals with obesity present with lower fasting plasma concentrations of ghrelin and lower postprandial secretion of GLP-1 (Dirksen et al., 2019; le Roux, Aylwin, et al., 2006), PYY (Berlakovich, Obeid, & El Odaib, 2007; El Helou, Obeid, & Rehfeld, 1998), and even lower postprandial fullness (Adam et al., 2015; Zwirska-Korczala et al., 2007) have looked into this effect. Considering this, little is known regarding potential alterations in the appetite control system among obesity classes, and if the secretion of GI hormones changes with increasing BMI. To our knowledge, few studies have compared obesity classes against a control group without obesity.

2. Materials and methods

2.1. Study design

This is a cross sectional case control study, where individuals with different obesity classes were compared among themselves, and then against a control group without obesity.

2.2. Participants

This manuscript reports a secondary analysis of the “Weight loss maintenance and compensatory mechanisms activated with a very-low energy diet (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. The main findings have already been published (Nymo et al., 2017, 2018). Adults (18-65 year) with obesity (BMI: ≥30 kg/m²), and a control group (BMI: 18.5-29.9 kg/m²) were recruited via newspaper advertising serving the community of Trondheim, Norway. All participants provided written informed consent before commencement. At recruitment, all participants were required to be weight stable (< 2 kg body weight change over the past 3 months), not currently dieting to lose weight, and have a sedentary lifestyle (walking < 150 min/week of physical activity of at least moderate intensity) (Haskell et al., 2007). The study excluded pregnant or breastfeeding women and those with clinically significant illnesses, including diabetes, previous weight loss surgery, and/or taking medication known to affect appetite or induce weight loss.

2.3. Outcome variables

The following measurements were performed:

2.3.1. Body weight and composition

Body weight- and composition (fat mass (FM) and fat free mass (FFM)) were measured in the fasting state with air-displacement plethysmography (BodPod, COSMED, Rome, Italy).

2.3.2. Appetite measures

Subjective appetite ratings (hunger, fullness, desire to eat (DTE), and prospective food consumption (PFC)) were measured using a 100-mm visual analog scale (VAS) (Stubbs et al., 2000). VAS and blood samples were collected in the fasting state, immediately after a fixed breakfast meal (VAS only), (2512 kJ [600 kcal]: 17% protein, 35% fat, and 48% carbohydrates) and every 30 min for a period of 2.5 h. Plasma samples were analyzed for acylated ghrelin, active GLP-1, total PYY and insulin using a Human Metabolic Hormone Magnetic Bead Panel (LINCOplex Kit, Millipore, St Louis, MO). The cross-reactivity between antibodies and any of the other analytes in this panel is non-detectable or negligible. CCK was analyzed using an “in-house” radioimmunoassay (Rehfeld, 1998). Intra- and inter-assays CV were <10% and <20% for acylated ghrelin, GLP-1 and PYY; <10% and <15% for insulin and <5% and <15% for CCK, respectively. Blood samples were collected in 4 ml EDTA-coated tubes. One milliliter of whole blood was then transferred into a micro tube and a 20 μL mixture of inhibitor (10 μL of Pefabloc [Roche Diagnostic, Germany] + 10 μL dipeptidyl-peptidase IV inhibitor [Merck Millipore, Germany]) was added. For CCK, 500 KIU aprotinin (DSM, Coatech AB, Kaiseraugst, Switzerland)/mL whole blood was added to the EDTA tube. Samples were then centrifuged at 2106 RCF for 10 min at 18 °C and the plasma frozen at −80 °C until further analysis. All the samples from the same participant were analyzed in the same plate. The analyses were performed by the same technician, except for CCK, which was analyzed at the University of Copenhagen, Denmark.

2.3.3. Physical activity

Habitual physical activity levels were measured with SenseWear armbands (BodyMedia, Pittsburgh, PA, USA) for 7-days prior to the assessments. Data was considered valid if participants wore the device for ≥4 days, including at least 1 weekend day, on more than 95% (22.8 h/day) of the time (Jakicic et al., 2004). Total physical activity was defined as time (minutes/day) spent on activities >1.5 metabolic equivalents.

2.4. Power calculation

Several studies have shown that individuals with obesity have a lower postprandial GLP-1 response compared to those with normal weight (Dirksen et al., 2019; le Roux, Aylwin, et al., 2006; Meyer-Gerspach et al., 2014). However, there are no studies comparing GLP-1 AUC among obesity classes. We hypothesized that individuals with obesity class I, II and –III would have a 10, 20 and 30% lower GLP-1 AUC, respectively, compared to controls without obesity (1533, 1226 and 858 min*pmol/L, respectively) (DeBenedictis et al., 2020). For a power of 80%, a significance level of 0.05, and assuming a within group variance of 640,000 min*pmol/L, 87 participants (29 in each group) would be required.

2.5. Statistical analysis

The statistical analysis was carried out using SPSS, version 26 (SPSS Inc., Chicago, IL). Data are presented as estimated marginal means ± SEM, unless otherwise stated. Due to the large number of tests, the significance level was reduced to P < 0.01, in order to account for increased risk of type-1 error. Two separate general linear models were performed to compare GI hormones and subjective appetite ratings
among different BMI classes, presented as estimated marginal means. One model compared individuals with obesity class I, II, and III, and a second model compared individuals with obesity (and its subclasses) with controls (a total of 6 comparisons). Residuals were checked for normality with Shapiro-Wilk test and did not deviate significantly from normality. Plasma concentration of GI hormones and appetite ratings were analyzed as dependent variables, with BMI group as a fixed factor. Covariates known to affect appetite (age, sex and physical activity) were added to both models, and Bonferroni correction was used for post-hoc pairwise comparisons. The trapezoidal rule was applied to calculate total area under the curve (tAUC) and incremental area under the curve (iAUC) from 0 to 150 min. A linear mixed model was run to look at changes in plasma concentrations of GI hormones over the sampling time period (0, 30, 60, 90, 120 and 150 min) in each group, using Bonferroni correction for post-hoc pairwise comparisons. Spearman correlation was used to investigate the association between BMI and the different appetite variables.

3. Results

The general characteristics of the participants are presented in Table 1. 143 participants were included in the analysis; 98 individuals with obesity (OB), further divided into class I (OBI), –II (OBI) and –III (OBIII) (BMI: 32.9 ± 0.3 kg/m², 37.2 ± 0.3 kg/m² and 43.1 ± 0.4 kg/m², respectively) and 45 control individuals without obesity (BMI: 24.6 ± 0.6 kg/m²). There were no significant differences in age or sex distribution between groups. FM (kg) differed between all groups, with FM increasing with BMI category (P < 0.001, for all). FFM (kg) was greater in the obesity group compared to controls (P < 0.001). Obesity class III had a greater FFM (kg) compared to the other two classes (P < 0.001, P = 0.003 and P = 0.006, for controls, OBI and OBIII respectively). OBI had borderline higher FFM (kg) compared to controls (P = 0.011). FFM (%) decreased with increasing BMI and was lower in all obesity classes compared to controls (P < 0.01, for all). There was no difference in total physical activity duration among obesity classes, but the obesity group had a lower total physical activity duration compared to controls (P = 0.005), and borderline lower in OBI compared to controls (P = 0.011).

Table 2 shows mean basal and postprandial concentrations of GI hormones, and plasma concentrations over time can be seen in Fig. 1. No differences were found among classes of obesity in either basal or postprandial plasma ghrelin concentrations. However, the postprandial suppression in ghrelin secretion was absent in obesity class III. All obesity classes presented with lower basal (P < 0.001, for all) and postprandial (tAUC; P < 0.001, for all) (borderline for iAUC in OB, P = 0.013) ghrelin concentration compared controls.

No differences were found for basal or postprandial concentrations of GLP-1 among obesity classes. Compared to basal values, postprandial secretion of GLP-1 was only elevated at minute 30 and 90 in OBI, and at no postprandial timepoint in OBIII. GLP-1 iAUC was lower in all obesity classes, and the obesity group, compared to controls (P < 0.001, for all). All obesity classes reached peak GLP-1 concentrations after 30 min whilst, controls reached their peak at 120 min after the fixed meal.

There were no differences among obesity classes in basal or postprandial secretion of PYY. PYY returned to basal concentrations at 150 min in OBI, while OBI and OBIII did not experience any postprandial increase in PYY. Individuals with obesity had lower PYY concentrations throughout the postprandial period compared to controls. Basal concentration of PYY was lower in the obesity group (P = 0.006) compared to controls. tAUC for PYY was lower in OBI, OBIII and OBIIII (P = 0.009, P < 0.001 and P < 0.001, respectively) and the obesity group (P < 0.001), as well as OBI for OBI and the obesity group compared to controls (P = 0.001 and P < 0.001, respectively). The different obesity classes reached peak PYY concentrations at minute 60, whilst controls reached their peak 120 min after the fixed meal.

No differences were found for basal or postprandial CCK concentration among obesity classes, or between obesity classes and controls. All groups experienced a similar postprandial curve, with the exception of OBI where the CCK response was delayed and shortened (only greater than basal concentrations between 60 and 120 min after the fixed meal).

OBII and OBIII had greater basal insulin concentration compared to controls (P = 0.007 and P < 0.001, respectively). Postprandial insulin (iAUC and tAUC) was greater in OBIIII versus OBI, OBI and controls (P < 0.001, P < 0.01 and P < 0.01, respectively). Compared to basal concentrations, insulin was elevated throughout all sample timepoints in all obesity classes, while controls returned to basal concentrations 2.5 h after the fixed meal.

Fasting and postprandial ratings of appetite are shown in Table 3 and postprandial ratings over time can be seen in Fig. 2. There were no differences among obesity classes in fasting or postprandial ratings of hunger, fullness, DTE or PFC. Postprandial PFC (iAUC) was greater in OBI compared to OBIII and OBIII (P = 0.009, P < 0.001 and P < 0.001, respectively). The different obesity classes reached peak PYY concentrations at minute 60, whilst controls reached their peak 120 min after the fixed meal.

Associations between BMI, plasma concentration of GI hormones, and appetite ratings can be seen in Table 4. Significant inverse associations with BMI were found for basal and postprandial ghrelin (P < 0.001, for both), postprandial GLP-1 plasma concentration (P < 0.001)

Table 1

|         | n   | Control | OB | OB I | OB II | OB III |
|---------|-----|---------|----|------|-------|--------|
| Age (years) | 45  | 98      | 35 | 44   | 19    |
| Females (%) | 58  | 55      | 49 | 66   | 42    |
| BMI (kg/m2) | 24.6±2.7 | 36.8±4.1 | 32.9±1.4 | 40.9±6.6 | 45.8±5.5 |
| FM (%) | 30.1±7.1 | 44.3±6.3 | 40.9±6.6 | 45.8±5.5 | 46.9±5.1 |
| (females) | (33.2±0.9 | 48.1±0.6 | 46.2±1.1 | 48.5±0.8 | 50.7±1.6 |
| (males)  | (25.9±1.2 | 39.5±0.9 | 35.8±1.2 | 40.4±1.3 | 44.2±1.4 |
| FM (kg)  | 22.5±6.7 | 48.8±11.1 | 40.1±6.3 | 50.1±6.4 | 61.8±12.3 |
| (females) | (23.1±1.6 | 49.6±1.1 | 42.2±1.6 | 51.1±1.2 | 59.8±2.4 |
| (males)  | (22.1±2.9 | 47.7±1.8 | 38.0±2.1 | 47.9±2.3 | 63.3±2.5 |
| FFM (%)  | 69.9±7.1 | 55.7±6.3 | 59.1±6.6 | 54.1±5.5 | 53.1±5.1 |
| (females) | (66.7±0.9 | 51.9±0.6 | 53.8±1.1 | 51.5±0.8 | 49.3±1.6 |
| (males)  | (74.1±1.2 | 60.5±0.9 | 64.1±1.2 | 59.6±1.3 | 55.8±1.5 |
| FFM (kg) | 52.2±10.7 | 61.3±11.6 | 58.7±11.4 | 59.6±10.2 | 69.7±11.7 |
| (females) | (44.9±1.0 | 53.0±0.7 | 48.9±1.1 | 54.0±0.8 | 57.9±1.5 |
| (males)  | (62.2±2.1 | 71.6±1.3 | 67.9±1.9 | 71.1±2.1 | 78.4±2.4 |
| Total PA (min/day) | 342±126 | 276±103 | 301±118 | 257±97 | 271±77 |

Data presented as estimated marginal means ± SEM. BMI = body mass index. FM = fat mass. FFM = fat free mass. OB = obesity. PA = physical activity. Mean values with equal superscript letters denote significant differences between groups (P < 0.01) after Bonferroni adjustment.
Table 2
Mean basal and postprandial concentrations of GI hormones.

|                      | Controls | OB     | OB I    | OB II   | OB III  |
|----------------------|----------|--------|---------|---------|---------|
| Basal ghrelin (pmol/l) | 72 ± 6 abc | 30 ± 4 a | 33 ± 6 b | 31 ± 5 c | 25 ± 9 d |
| Ghrelin tAUC (pmol/l/min) | 8054 ± 591 abc | 3095 ± 379 a | 3453 ± 618 b | 2938 ± 578 c | 2708 ± 912 d |
| Ghrelin iAUC (pmol/l/min) | −2712 ± 400 a | −1494 ± 257 a | −1424 ± 419 b | −1721 ± 392 c | −1067 ± 618 d |
| Basal GLP-1 (pmol/l) | 1 ± 1 | 3 ± 1 | 2 ± 1 | 3 ± 1 | 3 ± 2 |
| GLP-1 tAUC (pmol/l/min) | 1028 ± 151 | 691 ± 97 | 736 ± 158 | 633 ± 148 | 742 ± 233 |
| GLP-1 iAUC (pmol/l/min) | 839 ± 69 abc | 270 ± 45 a | 372 ± 72 b | 198 ± 67 c | 227 ± 106 d |
| Basal PYY (pmol/l) | 21 ± 3 a | 11 ± 2 a | 12 ± 3 | 11 ± 3 | 8 ± 5 |
| PYY tAUC (pmol/l/min) | 4522 ± 409 abc | 2011 ± 275 a | 2525 ± 445 b | 1807 ± 421 c | 1447 ± 622 d |
| PYY iAUC (pmol/l/min) | 1383 ± 200 abc | 386 ± 134 a | 616 ± 218 | 203 ± 207 f | 340 ± 305 d |
| Basal CCK (pmol/l) | 408 ± 29 | 400 ± 19 | 409 ± 30 | 403 ± 28 | 370 ± 45 |
| CCK tAUC (pmol/l/min) | 284 ± 23 | 242 ± 15 | 257 ± 24 | 257 ± 23 e | 170 ± 36 |
| CCK iAUC (pmol/l/min) | 3389 ± 597 abcd | 6351 ± 396 a | 5524 ± 625 | 6219 ± 584 f | 8526 ± 922 d |
| Basal insulin (pmol/l) | 2043,605 ± 344,834 abcd | 3,677,969 ± 239,476 a | 3,275,226 ± 360,896 b | 3,238,669 ± 337,605 c | 5,696,987 ± 552,872 d |
| Insulin tAUC (pmol/l/min) | 2,043,605 ± 344,834 abcd | 3,677,969 ± 239,476 a | 3,275,226 ± 360,896 b | 3,238,669 ± 337,605 c | 5,696,987 ± 552,872 d |
| Insulin iAUC (pmol/l/min) | 1,543,046 ± 294,514 ad | 2,679,982 ± 204,741 a | 2,443,018 ± 398,231 f | 2,211,975 ± 288,994 g | 4,405,207 ± 455,112 d |

Data presented as estimated marginal means ± SEM and adjusted for age, sex and total physical activity duration, and pairwise comparisons for the general mixed model. Accounting for multiplicity, significance level was set to P < 0.01. Conversion from metric to SI units has been made in Table 2 and Fig. 1 and is as follows: ghrelin pg/ml × 0.3 = pmol/l, GLP-1 pg/ml × 0.33 = pmol/l, PYY pg/ml × 0.25 = pmol/l, and insulin pg/ml × 6 = pmol/l. CCK: cholecystokinin. GLP-1: glucagon-like peptide-1. iAUC: incremental area under the curve. OB: obesity. PYY: peptide YY. tAUC: total area under the curve. Mean values with equal superscript letter denote significant differences between groups (P < 0.01) after Bonferroni adjustment.
mixed model. Accounting for multiplicity, significance level was set to $P < 0.01$ after Bonferroni adjustment.

Prospective food consumption. tAUC: total area under the curve. Mean values display an abnormal postprandial secretion of GI hormones compared to controls, and postprandial curves are somewhat dissimilar. Basal and postprandial secretion of ghrelin was lower in all obesity classes compared to controls, with no postprandial ghrelin suppression, from basal values, in obesity class III. Postprandial GLP-1, PYY and CCK responses were lower, or to some extent absent, in some obesity classes compared to controls. Basal and postprandial concentrations of insulin increased progressively across obesity classes. These findings were supported by correlational analyses, which showed inverse associations between BMI and basal and postprandial ghrelin, postprandial GLP-1, and basal and postprandial PYY, as well as positive associations between BMI and basal and postprandial insulin.

Studies looking at potential differences among obesity classes are few. We are aware of only two studies who have addressed this issue. Acosta and colleagues (Acosta et al., 2015) examined associations of GI traits with obesity before and after a nutrient drink test and reported a borderline inverse association between BMI and fasting ghrelin plasma concentrations ($P = 0.063$). However, ghrelin was only measured in overweight subjects and individuals with obesity class I and II/III obesity, and not in subjects with normal weight. Zwirska-Korczala and colleagues (Zwirska-Korczala et al., 2007) analyzed GI hormones in lean control women, women with moderate obesity and severe obesity (BMI: $23.2 \pm 0.7, 34.9 \pm 0.9$ and $46.9 \pm 1.6$ kg/m$^2$, respectively). They reported lower fasting total ghrelin in both obesity groups compared to controls. After a standard mixed meal (527 kcal), ghrelin concentration did not change in either obesity group, while values dropped and remained low in controls. Further, postprandial ghrelin concentration was lower in women with moderate obesity compared to women with severe obesity. This is in line with the present analysis showing no postprandial change in ghrelin plasma concentration in obesity class III.

### 4. Discussion

The aim of this study was to compare plasma concentrations of GI hormones and subjective appetite ratings among obesity classes, as well as between obesity classes and controls. Our results show that GI hormones and subjective ratings of appetite generally do not differ among classes of obesity (with the exception of insulin), but some obesity classes display an abnormal postprandial secretion of GI hormones compared to controls, and postprandial curves are somewhat dissimilar. Basal and postprandial secretion of ghrelin was lower in all obesity classes compared to controls, with no postprandial ghrelin suppression, from basal values, in obesity class III. Postprandial GLP-1, PYY and CCK responses were lower, or to some extent absent, in some obesity classes compared to controls. Basal and postprandial concentrations of insulin increased progressively across obesity classes. These findings were supported by correlational analyses, which showed inverse associations between BMI and basal and postprandial ghrelin, postprandial GLP-1, and basal and postprandial PYY, as well as positive associations between BMI and basal and postprandial insulin.

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### Table 3

Mean fasting and postprandial scores for appetite ratings.

|                         | Controls | OB         | OB I        | OB II       | OB III      |
|-------------------------|----------|------------|-------------|-------------|-------------|
| Fasting hunger (mm)     | 45 ± 4   | 39 ± 2     | 40 ± 4      | 39 ± 4      | 37 ± 6      |
| Hunger tAUC (mm*min)    | 2829 ±   | 3436 ±    | 3995 ±      | 3153 ±      | 2959 ±      |
| Hunger IAUC (mm*min)    | 413 ±    | 259 ±     | 421 ±       | 391 ±       | 600 ±       |
| Hunger IAUC (mm*min)    | −3870 ±  | −2451 ±   | −2060 ±     | −2760 ±     | −2515 ±     |
| Fasting fullness (mm)   | 22 ± 3   | 21 ± 2     | 18 ± 3      | 24 ± 3      | 18 ± 5      |
| Fullness tAUC (mm*min)  | 8597 ±   | 8969 ±    | 8552 ±      | 9125 ±      | 9458 ±      |
| Fullness IAUC (mm*min)  | 471 ±    | 293 ±     | 480 ±       | 446 ±       | 684 ±       |
| Fullness IAUC (mm*min)  | 5336 ±   | 5830 ±    | 5796 ±      | 5490 ±      | 6713 ±      |
| Fullness IAUC (mm*min)  | 570 ±    | 356 ±     | 581 ±       | 540 ±       | 828 ±       |
| Fasting DTE (mm*min)    | 46 ± 4   | 47 ± 2     | 49 ± 4      | 48 ± 4      | 41 ± 5      |
| DTE tAUC (mm*min)       | 2955 ±   | 4229 ±    | 4759 ±      | 3959 ±      | 3783 ±      |
| DTE IAUC (mm*min)       | 447 ±    | 280 ±     | 447 ±       | 424 ±       | 650 ±       |
| DTE IAUC (mm*min)       | −3893 ±  | −2811 ±   | −2532 ±     | −3210 ±     | −2433 ±     |
| Fasting PFC (mm*min)    | 64 ± 4   | 59 ± 2     | 63 ± 4      | 56 ± 3      | 61 ± 5      |
| PFC tAUC (mm*min)       | 5187 ±   | 6623 ±    | 7043 ±      | 6046 ±      | 7137 ±      |
| PFC IAUC (mm*min)       | 576 ±    | 360 ±     | 587 ±       | 546 ±       | 837 ±       |
| PFC IAUC (mm*min)       | −4382 ±  | −2278 ±   | −2440 ±     | −2267 ±     | −1967 ±     |
| PFC IAUC (mm*min)       | 499 ±    | 318 ±     | ± 509       | ± 473       | ± 725       |

Data presented as estimated marginal means ± SEM and adjusted for age, sex and total physical activity duration, and pairwise comparisons for the general mixed model. Accounting for multiplicity, significance level was set to $P < 0.01$. DTE: desire to eat. IAUC: incremental area under the curve. OB: obesity. PFC: prospective food consumption. tAUC: total area under the curve. Mean values display an abnormal postprandial secretion of GI hormones compared to controls, and postprandial curves are somewhat dissimilar. Basal and postprandial secretion of ghrelin was lower in all obesity classes compared to controls, with no postprandial ghrelin suppression, from basal values, in obesity class III. Postprandial GLP-1, PYY and CCK responses were lower, or to some extent absent, in some obesity classes compared to controls. Basal and postprandial concentrations of insulin increased progressively across obesity classes. These findings were supported by correlational analyses, which showed inverse associations between BMI and basal and postprandial ghrelin, postprandial GLP-1, and basal and postprandial PYY, as well as positive associations between BMI and basal and postprandial insulin.

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### Fig. 2

a-d. Mean fasting and postprandial appetite ratings over time.

Data presented as estimated marginal means ± SEM and adjusted for age, sex and total physical activity duration. Mean fasting and postprandial ratings of appetite in subgroups of obesity and controls. VAS: visual analogue scale.

as well as basal and postprandial PYY ($P = 0.003$, $P < 0.001$ and $P = 0.002$, respectively). Basal and postprandial insulin secretion were also positively associated with BMI ($P < 0.001$, for all).
hormones after a standardized solid fixed meal and found similar results, et al., 2006; Toft-Nielsen et al., 2001, have measured responses of GI PYY: peptide YY. tAUC: total area under the curve.

Correlation analysis between BMI and plasma concentration of GI hormones, and BMI and appetite ratings.

| BMI          | Spearman correlation coefficient (rho) | P    |
|--------------|----------------------------------------|------|
| Basal ghrelin| -0.531                                 | <0.001|
| Ghrelin tAUC | -0.536                                 | <0.001|
| Ghrelin IAUC | 0.376                                  | 0.003|
| Basal GLP-1  | -0.222                                 | 0.011|
| GLP-1 tAUC   | -0.388                                 | <0.001|
| GLP-1 IAUC   | -0.570                                 | <0.001|
| Basal PYY    | -0.294                                 | 0.003|
| PYY tAUC     | -0.462                                 | <0.001|
| PYY IAUC     | -0.308                                 | 0.002|
| Basal CCK    | 0.142                                  | 0.104|
| CCK tAUC     | -0.119                                 | 0.175|
| CCK IAUC     | -0.222                                 | 0.011|
| Basal insulin| 0.570                                  | <0.001|
| Insulin tAUC | 0.622                                  | <0.001|
| Insulin IAUC | 0.546                                  | <0.001|
| Fasting hunger| -0.183                                 | 0.029|
| Fasting CCK  | 0.102                                  | 0.606|
| Fasting IAUC | 0.135                                  | 0.108|
| Fasting fullness| 0.019                                 | 0.819|
| Fullness tAUC| 0.113                                  | 0.178|
| Fullness IAUC| 0.016                                  | 0.853|
| Fasting DTE  | -0.112                                 | 0.182|
| DTE tAUC     | 0.041                                  | 0.659|
| DTE IAUC     | 0.142                                  | 0.092|
| Fasting PFC  | -0.076                                 | 0.369|
| PFC tAUC     | 0.061                                  | 0.472|
| PFC IAUC     | 0.206                                  | 0.014|

Correlation is significant at the level 0.01. BMI: body mass index. CCK: cholecystokinin. DTE: desire to eat. GLP-1: glucagon-like peptide 1. IAUC: incremental area under the curve. P: p-value. PFC: prospective food consumption. PYY: peptide YY. tAUC: total area under the curve.

and an inverse relationship between BMI and both basal and postprandial plasma acylated ghrelin.

The findings of a weaker and earlier postprandial GLP-1 peak in individuals with obesity (and absence in obesity class III), as well as an inverse association between BMI and postprandial GLP-1 found in the present study are in contrast with those of Acosta and colleagues. They reported a higher GLP-1 peak in overweight, and obesity class I and class II/III compared to subjects with normal weight (Acosta et al., 2015). It is interesting to note that, the present study, and others (le Roux, Aylwin, II/III compared to subjects with normal weight (Acosta et al., 2015). It is interesting to note that, the present study, and others (M.I. Aukan et al., 2006; Toft-Nielsen et al., 2001; Zwirska-Korczala et al., 2007) have measured responses of GI hormones after a standardized solid fixed meal and found similar results, whereas PYY from dipeptidyl peptidase-4 metabolism and has been shown to inhibit food intake (Kuhre, Wever-Albrechtsen, Hartmann, Deacon, & Holst, 2015; Sloth, Davidsen, Holst, Flint, & Astrup, 2007).

BMI was associated with borderline higher postprandial concentrations in overweight and obesity. However, CCK was not assessed in subjects with normal weight in that study. Thus conclusions regarding CCK secretion remain controversial, likely due to methodological issues (Rehfeld, 2020).

Conflicting results among studies could be due to differences in energy- and macronutrient loads, gastric emptying, or other factors. For example, the energy, macronutrient composition, and physical form (solid versus liquid) of the test meal, as well as the proportion of the individual energy requirements covered by the test meal, are likely to affect the postprandial secretion of the different GI hormones (Mourao, Bressan, Campbell, & Mattes, 2007; Tischmann et al., 2019).

Women with severe obesity had been shown to have lower basal and postprandial CCK secretion compared to both women with moderate obesity and lean (Zwirska-Korczala et al., 2007). The present analysis found a delayed and shortened postprandial CCK response in individuals with obesity class III, which deviated from the other groups. Contrarily, in the study of Acosta and colleagues (Acosta et al., 2015), BMI was associated with borderline higher peak CCK concentrations in overweight and obesity. However, CCK was not assessed in subjects with normal weight in that study. Thus conclusions regarding CCK secretion remain controversial, likely due to methodological issues (Rehfeld, 2020).

Positive relationships between BMI and both basal and postprandial insulin secretion are well established (Bagdade et al., 1967), and the present analysis replicated this. Contrarily, Zwirska-Korczala and colleagues (Zwirska-Korczala et al., 2007) found no differences in basal concentrations of insulin between groups. However, and similar to the present analysis, insulin concentrations remained elevated throughout the postprandial period in both women with moderate and severe obesity, while in controls they returned to basal values at the end of the 2 h period. Of note, this study was carried out in women with obesity and the metabolic syndrome.

Even though infusions of GI hormones have been shown to affect eating behavior in both individuals with obesity and normal weight in laboratory settings (Batterham et al., 2002; de Graaf, Blom, Sneeds, Stafleu, & Hendriks, 2004; Druce et al., 2005; Flint, Raben, Astrup, & Holst, 1998; le Roux, Batterham, et al., 2006), it needs to be emphasized that the relationship between plasma concentration of GI hormones and subjective appetite ratings is highly complex (Crum, Corbin, Brownell, & Salovey, 2009). Individuals with obesity need to eat large meals than individuals with a normal weight, under conditions of weight stability. It remains, therefore, to be determined if the impaired satiety response in individuals with obesity, particularly in those with obesity class III, seen in the present study, and others (le Roux, Aylwin, et al., 2006; Toft-Nielsen et al., 2001; Zwirska-Korczala et al., 2007) which use a fixed test meal across BMI groups, remains when participants are allowed to eat/drink until satiation, as in Acosta et al. study (Acosta et al., 2015).

Positive relationships between BMI and both basal and postprandial insulin secretion are well established (Bagdade et al., 1967), and the present analysis replicated this. Contrarily, Zwirska-Korczala and colleagues (Zwirska-Korczala et al., 2007) found no differences in basal concentrations of insulin between groups. However, and similar to the present analysis, insulin concentrations remained elevated throughout the postprandial period in both women with moderate and severe obesity, while in controls they returned to basal values at the end of the 2 h period. Of note, this study was carried out in women with obesity and the metabolic syndrome. However, CCK secretion compared to controls in the present analysis. Zwirska-Korczala and colleagues (Zwirska-Korczala et al., 2007) reported basal PYY was seen in participants with severe obesity after a liquid meal, (Meyer-Gerspach et al., 2014). This is in line with the present analysis showing no postprandial total PYY response in individuals with obesity class II and III after the fixed breakfast meal. BMI was also negatively correlated with postprandial PYY concentrations. The absence of significant findings among obesity classes in the present analysis might result from lack of power, given that we did not have enough participants with obesity class II to reach the desired power. Also, differences in the hormonal fractions measured might affect results, and thus limit comparisons among studies. Total PYY is a measure of secretion, whereas PYY3-36 results from dipeptidyl peptidase-4 metabolism and has been shown to inhibit food intake (Kuhre, Wever-Albrechtsen, Hartmann, Deacon, & Holst, 2015; Sloth, Davidsen, Holst, Flint, & Astrup, 2007). Women with severe obesity have been shown to have lower basal and postprandial CCK secretion compared to both women with moderate obesity and lean (Zwirska-Korczala et al., 2007). The present analysis found a delayed and shortened postprandial CCK response in individuals with obesity class III, which deviated from the other groups. Contrarily, in the study of Acosta and colleagues (Acosta et al., 2015), BMI was associated with borderline higher peak CCK concentrations in overweight and obesity. However, CCK was not assessed in subjects with normal weight in that study. Thus conclusions regarding CCK secretion remain controversial, likely due to methodological issues (Rehfeld, 2020).
Roux, Aylwin, et al., 2006) reported that individuals with obesity exhibited a lower postprandial GLP-1 response, no postprandial rise in PYY, and lower ratings of fullness after a fixed meal compared to controls. In another study by the same group (le Roux, Batterham, et al., 2006), attenuated postprandial PYY response was matched by a lower perception of postprandial fullness in individuals with obesity. In line with this, Acosta and colleagues (Acosta et al., 2015) showed that obesity was associated with decreased satiation and lower PYY peak (Acosta et al., 2015). Altogether, these results indicate reduced satiety and stronger drive to eat in individuals with obesity. Further, a study by Maier and colleagues (Maier et al., 2008) showed that changes in ghrelin and PYY plasma concentrations were correlated with hunger and fullness ratings, respectively, but only in individuals with a normal weight.

Strengths of the present analysis include its study design, which allowed for the evaluation of alterations in GI hormones and appetite ratings among obesity classes, as well as between obesity classes and controls, an issue that is under-investigated. Moreover, variables known to affect appetite were added to the models as covariates, and the significance level adjusted for multi-comparisons (Bonferroni) and the large number of variables tested. However, this study also has some limitations. First, a multiplex kit was used to measure hormonal concentrations, except for CCK. This method is likely to result in less accurate and precise measures compared to optimized assays for each hormone. Second, a classical 100 mm VAS were used to assess appetite ratings. This method is known to be less sensitive than generalized labeled magnitude scales when comparing differences between groups of individuals with healthy weight versus obesity (Bartoshuk, Duffy, Hayes, Moskowitz, & Snyder, 2006). Third, the energy load of the test meal was kept constant regardless of body weight. This ensured that the same nutritional stimulus was provided to all participants and did not account for individual energy needs. Fourth, participants in this study were not equally distributed among groups, the group of obesity class III was small, and controls included both subjects with normal weight and overweight. Last, this is a cross-sectional analysis comparing groups with different BMIs and, as such, a cause-effect relationship cannot be established.

5. Conclusions

In conclusion, the present analysis confirms that meal-stimulated secretion of GI hormones is impaired in individuals with obesity, especially those with obesity class III (and to some extent class II), where no postprandial decline in ghrelin concentration or increase in GLP-1 and PYY were seen. Additionally, obesity was associated with greater PFC. Altogether, this may strengthen the drive to eat and lead to overeating.

Disclosure summary

The authors have no conflict of interest to disclosure.

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Ethical statement

This manuscript reports a secondary analysis of the “Weight loss maintenance and compensatory mechanisms activated with a very-low energy diet (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.

Author contributions

C.M. and M.I.A formulated the research questions and designed the study. J.N.D, S.N, K.H.O, and G.A.B carried out the study. J.F.R analyzed CCK. M.I.A analyzed the data. All authors were involved in the writing of the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2022.105940.

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