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# 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.

## 1. Introduction

Obesity is a chronic, progressive, and relapsing disease (Bray, Kim, & Wilding, 2017) classified by body mass index (BMI) $\geq$30 kg/m$^2$. It is further divided into subclasses according to the degree of severity: class I (BMI: 30.0–34.9 kg/m$^2$), class II (BMI: 35.0–39.9 kg/m$^2$), and class III (BMI: $\geq$40.0 kg/m$^2$), 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 $\geq$40.0 kg/m$^2$ 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

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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, Al-Himadi, & Al-Odaib, 2007; El Helou, Obeid, & Olabi, 2019; le Roux, 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 and CCK (Batterham et al., 2003; Clamp, Hehir, Lambert, Beglinger, & Goedecke, 2015; le Roux, Aylwin, et al., 2006; Mans et al., 2015) compared to controls, although results are inconsistent (Brennan et al., 2012; Dirksen et al., 2019; El Helou et al., 2019; Federico et al., 2016; Rahat-Rozenbloom, Fernandes, Cheng, & Wolever, 2017). Moreover, basal and postprandial secretion of insulin has long been shown to be positively correlated with BMI (Bagdade, Bierman, & Porte, 1967). Even though no association between BMI and subjective ratings of appetite has been found (Gregersen et al., 2011; Painchaud Guerard et al., 2016), individuals with obesity report lower ratings of hunger in the fasted state (Wikarek, Chudek, Owczarek, & Olszanecka-Glinianowicz, 2014), reduced postprandial fullness (Adam & Westerterp-Plantenga, 2005; le Roux, Batterham, et al., 2006) and eat larger meals (Acosta et al., 2015; Meyer-Gerspach et al., 2014) compared to controls. Alterations in GI hormones may therefore play a key role in feeding behavior and obesity pathophysiology (Hansen, Andersen, Astrup, Blundell, & Sjodin, 2019; le; Roux, Batterham, et al., 2006; Steinert et al., 2017).

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 (Acosta et al., 2015; Zwirska-Korczala et al., 2007) have looked into this and results are so far inconclusive. Therefore, the main aim of this analysis was to compare GI hormones and subjective appetite ratings among obesity classes. A secondary aim was to compare obesity classes with controls 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: $\geq$30 kg/m$^2$), and a control group (BMI: 18.5–29.9 kg/m$^2$) 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 (engaging in <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 fasted 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, PO, USA) for 7-days prior to the assessments. Data was considered valid if participants wore the device for $\geq$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 (OBII) and –III (OBIII) (BMI: 32.9 ± 0.3 kg/m$^2$, 37.2 ± 0.3 kg/m$^2$ and 43.1 ± 0.4 kg/m$^2$, respectively) and 45 control individuals without obesity (BMI: 24.6 ± 0.6 kg/m$^2$). 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 OBII respectively). OBII 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 OBII 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 OBII, 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 OBII 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, OBII and OBIII ($P = 0.009$, $P < 0.001$ and $P < 0.001$, respectively) and the obesity group ($P < 0.001$), as well as iAUC for OBII 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 OBIII 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 OBIII versus OBII, 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 the obesity group compared to controls ($P = 0.001$).

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**
General characteristics of the participants.

| n | Control | OB | OB I | OB II | OB III |
|---|---|---|---|---|---|
| | 45 | 98 | 35 | 44 | 19 |
| **Age (years)** | 39.1 ± 11.1 | 42.4 ± 9.8 | 43.2 ± 9.9 | 42.8 ± 9.9 | 40.1 ± 9.4 |
| **Females (%)** | 58 | 55 | 49 | 66 | 42 |
| **BMI (kg/m2)** | 24.6 ± 2.7 | 36.8 ± 4.1 | 32.9 ± 1.4 | 37.2 ± 1.5 | 43.1 ± 3.2 |
| **FM (%)** | 30.1 ± 7.1 $^{abcd}$ | 44.3 ± 6.3 $^{a}$ | 40.9 ± 6.6 $^{bef}$ | 45.8 ± 5.5 $^{ce}$ | 46.9 ± 5.1 $^{df}$ |
| (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 $^{abcd}$ | 48.8 ± 11.1 $^{a}$ | 40.1 ± 6.3 $^{bef}$ | 50.1 ± 6.4 $^{ceg}$ | 61.8 ± 12.3 $^{dfg}$ |
| (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 $^{abcd}$ | 55.7 ± 6.3 $^{a}$ | 59.1 ± 6.6 $^{bef}$ | 54.1 ± 5.5 $^{ce}$ | 53.1 ± 5.1 $^{df}$ |
| (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 $^{ad}$ | 61.3 ± 11.6 $^{a}$ | 58.7 ± 11.4 $^{f}$ | 59.6 ± 10.2 $^{g}$ | 69.7 ± 11.7 $^{dfg}$ |
| (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 $^{a}$ | 276 ± 103 $^{a}$ | 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 [abcd] | 30 ± 4 [a] | 33 ± 6 [b] | 31 ± 5 [c] | 25 ± 9 [d] |
| **Ghrelin tAUC (pmol/l *min)** | 8054 ± 590 [abcd] | 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 | −1721 ± 392 | −1067 ± 618 |
| **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 [abcd] | 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 ± 408 [abcd] | 2011 ± 275 [a] | 2525 ± 445 [b] | 1807 ± 421 [c] | 1447 ± 622 [d] |
| **PYY iAUC (pmol/l *min)** | 1383 ± 200 [acd] | 386 ± 134 [a] | 616 ± 218 | 203 ± 207 [c] | 340 ± 305 [d] |
| **Basal CCK (pmol/l)** | 1 ± 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 |
| **CCK tAUC (pmol/l*min)** | 408 ± 29 | 400 ± 19 | 409 ± 30 | 403 ± 28 | 370 ± 45 |
| **CCK iAUC (pmol/l*min)** | 284 ± 23 | 242 ± 15 | 257 ± 24 | 257 ± 23 | 170 ± 36 |
| **Basal insulin (pmol/l)** | 3389 ± 597 [acd] | 6351 ± 396 [a] | 5524 ± 625 | 6219 ± 584 [c] | 8526 ± 922 [d] |
| **Insulin tAUC (pmol/l *min)** | 2,043,605 ± 344,834 [abcd] | 3,677,969 ± 239,476 [a] | 3,275,226 ± 360,896 [bf] | 3,238,669 ± 337,605 [cg] | 5,696,987 ± 532,872 [dfg] |
| **Insulin iAUC (pmol/l *min)** | 1,543,046 ± 294,514 [ad] | 2,679,982 ± 204,741 [a] | 2,443,018 ± 308,231 [f] | 2,211,975 ± 288,994 [g] | 4,405,207 ± 455,112 [dfg] |

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 x 0.33 = pmol/l, PYY pg/ml × 0.25 = pmol/l, and insulin pg/ml x 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.

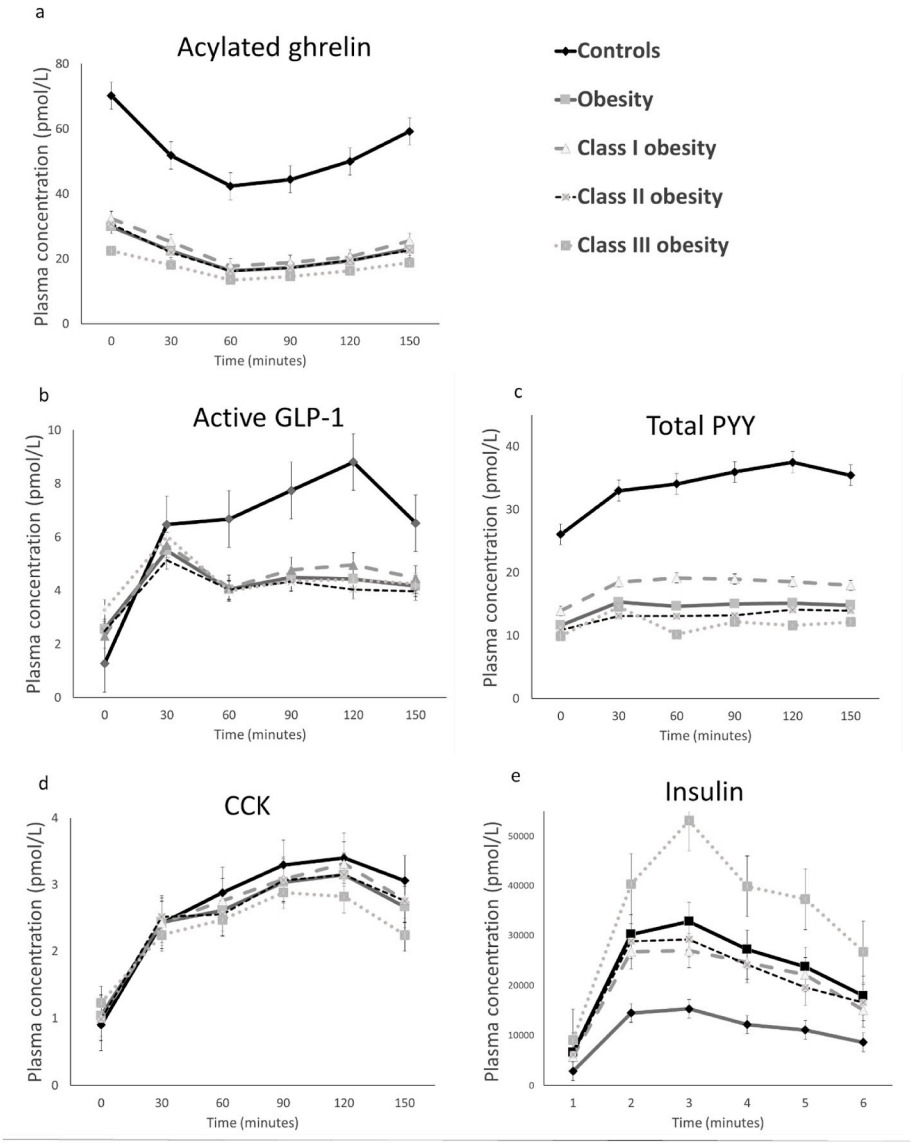


**Fig. 1.** a–e. Mean basal and postprandial plasma concentrations of gastrointestinal hormones over time.
Data presented as estimated marginal means ± SEM and adjusted for age, sex and total physical activity duration. AG: acylated ghrelin. CCK: cholecystokinin. GLP-1: glucagon-like peptide-1. PYY: peptide YY. Mean basal and postprandial plasma concentrations of GI hormones are shown in subgroups of obesity and controls. 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 x 0.33 = pmol/L, PYY pg/mL × 0.25 = pmol/L, and insulin pg/mL x 6 = pmol/L.

**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 ± 413 | 3436 ± 259 | 3995 ± 421 | 3153 ± 391 | 2959 ± 600 |
| **Hunger iAUC (mm*min)** | −3870 ± 540 | −2451 ± 336 | −2060 ± 550 | −2760 ± 512 | −2515 ± 784 |
| **Fasting fullness (mm)** | 22 ± 3 | 21 ± 2 | 18 ± 3 | 24 ± 3 | 18 ± 5 |
| **Fullness tAUC (mm*min)** | 8597 ± 471 | 8969 ± 293 | 8552 ± 480 | 9125 ± 446 | 9458 ± 684 |
| **Fullness iAUC (mm*min)** | 5336 ± 570 | 5830 ± 356 | 5796 ± 581 | 5490 ± 540 | 6713 ± 828 |
| **Fasting DTE (mm)** | 46 ± 4 | 47 ± 2 | 49 ± 4 | 48 ± 4 | 41 ± 5 |
| **DTE tAUC (mm*min)** | 2955 ± 447 | 4229 ± 280 | 4759 ± 447 | 3959 ± 424 | 3783 ± 650 |
| **DTE iAUC (mm*min)** | −3893 ± 472 | −2811 ± 294 | −2532 ± 481 | −3210 ± 447 | −2433 ± 685 |
| **Fasting PFC (mm)** | 64 ± 4 | 59 ± 2 | 63 ± 4 | 56 ± 3 | 61 ± 5 |
| **PFC tAUC (mm*min)** | 5187 ± 576 | 6623 ± 360 | 7043 ± 587 | 6046 ± 546 | 7137 ± 837 |
| **PFC iAUC (mm*min)** | −4382 ± 499 [a] | 2278 ± 310 [a] | −2440 ± 509 | −2267 ± 473 | −1967 ± 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 with equal superscript letter denote significant differences between groups ($P < 0.01$) after Bonferroni adjustment.

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).

## 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.

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 ± 0.7, 34.9 ± 0.9 and 46.9 ± 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,

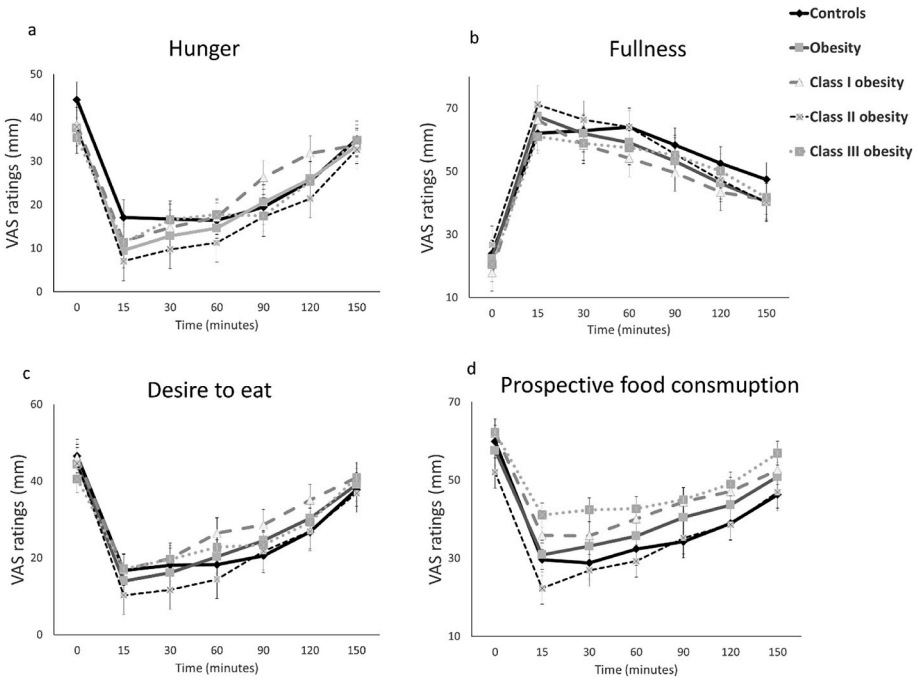


**Fig. 2.** a–d. Mean fasting and postprandial appetite ratings over time.
Data presented as estimated marginal means ± SEM and adjusted for age, sex and total physical activity duration. Mean fasting and postprandial ratings of appetite in subgroups of obesity and controls. VAS: visual analogue scale.

**Table 4**
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** | **<0.001** |
| 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 |
| Hunger tAUC | −0.102 | 0.606 |
| Hunger 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, et al., 2006; Toft-Nielsen et al., 2001), have measured responses of GI hormones after a standardized solid fixed meal and found similar results, while in Acosta and colleagues' study subjects drank a liquid meal until satiation (Acosta et al., 2015). The fact that obesity was associated with decreased satiation (measured by consumption of a higher volume (and kcal) of the nutrient drink to reach fullness) in addition to accelerated gastric emptying of liquids (Acosta et al., 2015), may explain why obesity was associated with higher GLP-1 peak in that study. Both the present and the forementioned studies measured the active form of GLP-1, but methodological differences still make it difficult to make direct comparisons (Heijboer, Frans, Lomecky, & Blankenstein, 2011).

Overall, individuals with obesity had lower basal and postprandial secretion of total PYY compared to controls in the present analysis. Zwirska-Korczala and colleagues (Zwirska-Korczala et al., 2007) reported basal $PYY_{3-36}$ to be reduced in obesity, particularly in those with severe obesity, compared to controls. Similarly, Acosta and colleagues (Acosta et al., 2015) found lower PYY postprandial peak in individuals with overweight and obesity class I, but not class II/III, when compared to subjects with normal weight. Given that individuals with class II/III obesity needed a larger volume of the nutrient drink test to reach fullness, it is surprising that their PYY postprandial peak concentrations did not differ from individuals with a normal weight. In another study by Meyer-Gerspach and collaborators, no postprandial increase in total 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 of lack of power, given that we did not have enough participants with obesity class III 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 $PYY_{3-36}$ results from dipeptidyl peptidase-4 metabolism and has been shown to inhibit food intake (Kuhre, WewerAlbrechtsen, 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).

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). Also, it needs to be acknowledged that individuals with obesity need to eat larger 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.

Even though infusions of GI hormones have been shown to affect eating behavior in both individuals with obesity and normal weight in laboratorial settings (Batterham et al., 2002; de Graaf, Blom, Smeets, 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, 2011). Individuals with obesity class II and III in the present sample seemed to have a more dysregulated hormonal response, but no differences in appetite ratings were seen. It is important to note in this connection that subjective appetite ratings represent an individual's interpretation of his/her feelings and motivations to eat rather than direct measures of the underlying physiological processes controlling eating (Blundell, 1979; Carroll, Kaiser, Franks, Deere, & Caffrey, 2007; Stubbs et al., 2000). Yet, obesity was associated with greater postprandial DTE in the present analysis. Also, Le Roux and colleagues (le

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 but 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.

## References

Acosta, A., Camilleri, M., Shin, A., Vazquez-Roque, M. I., Iturrino, J., Burton, D., et al. (2015). Quantitative gastrointestinal and psychological traits associated with obesity and response to weight-loss therapy. *Gastroenterology, 148*(3), 537–546. https://doi.org/10.1053/j.gastro.201411020. e534.

Adam, T. C. M., & Westerterp-Plantenga, M. S. (2005). Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects. *British Journal of Nutrition, 93*(6), 845–851.

Bagdade, J. D., Bierman, E. L., & Porte, D., Jr. (1967). The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *Journal of Clinical Investigation, 46*(10), 1549–1557. https://doi.org/10.1172/jci105646

Bartoshuk, L. M., Duffy, V. B., Hayes, J. E., Moskowitz, H. R., & Snyder, D. J. (2006). Psychophysics of sweet and fat perception in obesity: Problems, solutions and new perspectives. *Philosophical Transactions of the Royal Society of London B Biological Sciences, 361*(1471), 1137–1148. https://doi.org/10.1098/rstb.2006.1853

Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., et al. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. *New England Journal of Medicine, 349*(10), 941–948.

Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., et al. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature, 418*(6898), 650–654. https://doi.org/10.1038/nature00887

Blundell, J. (1979). *Hunger, appetite and satiety - constructs in search of identities. In Nutrition and Lifestyles* (pp. 21–42). London: Applied Science.

Bray, G. A., Kim, K. K., & Wilding, J. P. H. (2017). Obesity: A chronic relapsing progressive disease process. A position statement of the World Obesity Federation. *Obesity Reviews, 18*(7), 715–723. https://doi.org/10.1111/obr.12551

Brennan, I. M., Luscombe-Marsh, N. D., Seimon, R. V., Otto, B., Horowitz, M., Wishart, J. M., et al. (2012). Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 303*(1), G129–G140. https://doi.org/10.1152/ajpgi.00478.2011

Carroll, J. F., Kaiser, K. A., Franks, S. F., Deere, C., & Caffrey, J. L. (2007). Influence of BMI and gender on postprandial hormone responses. *Obesity, 15*(12), 2974–2983. https://doi.org/10.1038/oby.2007.355

Clamp, L., Hehir, A. P. J., Lambert, E. V., Beglinger, C., & Goedecke, J. H. (2015). Lean and obese dietary phenotypes: Differences in energy and substrate metabolism and appetite. *British Journal of Nutrition, 114*(10), 1724–1733.

Crum, A. J., Corbin, W. R., Brownell, K. D., & Salovey, P. (2011). Mind over milkshakes: Mindsets, not just nutrients, determine ghrelin response. *Health Psychology, 30*(4), 424–429. https://doi.org/10.1037/a0023467. discussion 430-421.

Daghestani, M. H., Ozand, P. T., Al-Himadi, A. R., & Al-Odaib, A. N. (2007). Hormonal levels of leptin, insulin, ghrelin, and neuropeptide Y in lean, overweight, and obese Saudi females. *Saudi Medical Journal, 28*(8), 1191–1197.

DeBenedictis, J. N., Nymo, S., Ollestad, K. H., Boyesen, G. A., Rehfeld, J. F., Holst, J. J., et al. (2020). Changes in the homeostatic appetite system After weight loss reflect a normalization toward a lower body weight. *Journal of Clinical Endocrinology & Metabolism, 105*(7), 1. https://doi.org/10.1210/clinem/dgaa202

Dirksen, C., Graff, J., Fuglsang, S., Rehfeld, J. F., Holst, J. J., & Madsen, J. L. (2019). Energy intake, gastrointestinal transit, and gut hormone release in response to oral triglycerides and fatty acids in men with and without severe obesity. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 316*(3), G332–G337. https://doi.org/10.1152/ajpgi.00310.2018

Druce, M. R., Wren, A. M., Park, A. J., Milton, J. E., Patterson, M., Frost, G., et al. (2005). Ghrelin increases food intake in obese as well as lean subjects. *International Journal of Obesity, 29*(9), 1130–1136. https://doi.org/10.1038/sj.ijo.0803001

El Helou, N., Obeid, O. A., & Olabi, A. (2019). Effect of meal acceptability on postprandial appetite scores and hormones of male participants with varied adiposity. *Obesity, 27*(10), 1627–1633. https://doi.org/10.1002/oby.22583

Federico, A., Dallio, M., Tolone, S., Gravina, A. G., Patrone, V., Romano, M., et al. (2016). Gastrointestinal hormones, intestinal microbiota and metabolic homeostasis in obese patients: Effect of bariatric surgery. *In Vivo, 30*(3), 321–330.

Flint, A., Raben, A., Astrup, A., & Holst, J. J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *Journal of Clinical Investigation, 101*(3), 515–520. https://doi.org/10.1172/jci990

de Graaf, C., Blom, W. A., Smeets, P. A., Stafleu, A., & Hendriks, H. F. (2004). Biomarkers of satiation and satiety. *American Journal of Clinical Nutrition, 79*(6), 946–961. https://doi.org/10.1093/ajcn/79.6.946

Gregersen, N. T., Møller, B. K., Raben, A., Kristensen, S. T., Holm, L., Flint, A., et al. (2011). Determinants of appetite ratings: The role of age, gender, BMI, physical activity, smoking habits, and diet/weight concern. *Food & Nutrition Research, 55*. https://doi.org/10.3402/fnr.v55i0.7028

Hall, K. D., & Guo, J. (2017). Obesity energetics: Body weight regulation and the effects of diet composition. *Gastroenterology, 152*(7), 1718–1727. https://doi.org/10.1053/j.gastro.2017.01.052. e1713.

Hansen, T. T., Andersen, S. V., Astrup, A., Blundell, J., & Sjodin, A. (2019). Is reducing appetite beneficial for body weight management in the context of overweight and obesity? A systematic review and meta-analysis from clinical trials assessing body weight management after exposure to satiety enhancing and/or hunger reducing products. *Obesity Reviews, 20*(7), 983–997. https://doi.org/10.1111/obr.12854

Haskell, W. L., Lee, I. M., Pate, R. R., Powell, K. E., Blair, S. N., Franklin, B. A., et al. (2007). Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Medicine & Science in Sports & Exercise, 39*(8), 1423–1434. https://doi.org/10.1249/mss.0b013e3180616b27

Heijboer, A. C., Frans, A., Lomecky, M., & Blankenstein, M. A. (2011). Analysis of glucagon-like peptide 1; what to measure? *Clinica Chimica Acta, 412*(13–14), 1191–1194. https://doi.org/10.1016/j.cca.2011.03.010

Jakicic, J. M., Marcus, M., Gallagher, K. I., Randall, C., Thomas, E., Goss, F. L., et al. (2004). Evaluation of the SenseWear pro armband??? to assess energy expenditure during exercise. *Medicine & Science in Sports & Exercise*, 897–904. https://doi.org/10.1249/01.Mss.0000126805.32659.43

Kuhre, R. E., Wewer Albrechtsen, N. J., Hartmann, B., Deacon, C. F., & Holst, J. J. (2015). Measurement of the incretin hormones: Glucagon-like peptide-1 and glucose-dependent insulinotropic peptide. *Journal of Diabetic Complications, 29*(3), 445–450. https://doi.org/10.1016/j.jdiacomp.2014.12.006

Maier, C., Riedl, M., Vila, G., Nowotny, P., Wolzt, M., Clodi, M., et al. (2008). Cholinergic regulation of ghrelin and peptide YY release may be impaired in obesity. *Diabetes, 57*(9), 2332–2340. https://doi.org/10.2337/db07-0758

Mans, E., Serra-Prat, M., Palomera, E., Sunol, X., & Clave, P. (2015). Sleeve gastrectomy effects on hunger, satiation, and gastrointestinal hormone and motility responses after a liquid meal test. *American Journal of Clinical Nutrition, 102*(3), 540–547. https://doi.org/10.3945/ajcn.114.104307

Meyer-Gerspach, A. C., Wolnerhanssen, B., Beglinger, B., Nessenius, F., Napitupulu, M., Schulte, F. H., et al. (2014). Gastric and intestinal satiation in obese and normal weight healthy people. *Physiology & Behavior, 129*, 265–271. https://doi.org/10.1016/j.physbeh.2014.02.043

Mourao, D. M., Bressan, J., Campbell, W. W., & Mattes, R. D. (2007). Effects of food form on appetite and energy intake in lean and obese young adults. *International Journal of Obesity, 31*(11), 1688–1695. https://doi.org/10.1038/sj.ijo.0803667

Nymo, S., Coutinho, S. R., Eknes, P. H., Vestbostad, I., Rehfeld, J. F., Truby, H., et al. (2018). Investigation of the long-term sustainability of changes in appetite after weight loss. *International Journal of Obesity, 42*(8), 1489–1499. https://doi.org/10.1038/s41366-018-0119-9

Nymo, S., Coutinho, S. R., Jorgensen, J., Rehfeld, J. F., Truby, H., Kulseng, B., et al. (2017). Timeline of changes in appetite during weight loss with a ketogenic diet. *International Journal of Obesity, 41*(8), 1224–1231. https://doi.org/10.1038/ijo.2017.96

Painchaud Guerard, G., Lemieux, S., Doucet, E., Pomerleau, S., & Provencher, V. (2016). Influence of nutrition claims on appetite sensations according to sex, weight status, and restrained eating. *Journal of Obesity*, 9475476, 2016.

Rahat-Rozenbloom, S., Fernandes, J., Cheng, J., & Wolever, T. M. S. (2017). Acute increases in serum colonic short-chain fatty acids elicited by inulin do not increase GLP-1 or PYY responses but may reduce ghrelin in lean and overweight humans. *European Journal of Clinical Nutrition, 71*(8), 953–958. https://doi.org/10.1038/ejcn.2016.249

Rehfeld, J. F. (1998). Accurate measurement of cholecystokinin in plasma. *Clinical Chemistry, 44*(5), 991–1001.

Rehfeld, J. F. (2020). Measurement of cholecystokinin in plasma with reference to nutrition related obesity studies. *Nutrition Research, 76*, 1–8. https://doi.org/10.1016/j.nutres.2020.01.003

le Roux, C. W., Aylwin, S. J., Batterham, R. L., Borg, C. M., Coyle, F., Prasad, V., et al. (2006). Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Annals of Surgery, 243*(1), 108–114. https://doi.org/10.1097/01.sla.0000183349.16877.84

le Roux, C. W., Batterham, R. L., Aylwin, S. J., Patterson, M., Borg, C. M., Wynne, K. J., et al. (2006). Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology, 147*(1), 3–8. https://doi.org/10.1210/en.2005-0972

Sloth, B., Davidsen, L., Holst, J. J., Flint, A., & Astrup, A. (2007). Effect of subcutaneous injections of PYY1-36 and PYY3-36 on appetite, ad libitum energy intake, and plasma free fatty acid concentration in obese males. *American Journal of Physiology. Endocrinology and Metabolism, 293*(2), E604–E609. https://doi.org/10.1152/ajpendo.00153.2007

Steinert, R. E., Feinle-Bisset, C., Asarian, L., Horowitz, M., Beglinger, C., & Geary, N. (2017). Ghrelin, CCK, GLP-1, and PYY(3-36): Secretory controls and physiological roles in eating and glycemia in health, obesity, and after RYGB. *Physiological Reviews, 97*(1), 411–463. https://doi.org/10.1152/physrev.00031.2014

Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., Elia, M., et al. (2000). 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. *British Journal of Nutrition, 84*(4), 405–415. https://doi.org/10.1017/s0007114500001719

Tischmann, L., Drummen, M., Gatta-Cherifi, B., Raben, A., Fogelholm, M., Hartmann, B., et al. (2019). Effects of a high-protein/moderate-carbohydrate diet on appetite, gut peptides, and endocannabinoids-A preview study. *Nutrients, 11*(10). https://doi.org/10.3390/nu11102269

Toft-Nielsen, M. B., Damholt, M. B., Madsbad, S., Hilsted, L. M., Hughes, T. E., Michelsen, B. K., et al. (2001). Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *Journal of Clinical Endocrinology & Metabolism, 86*(8), 3717–3723. https://doi.org/10.1210/jcem.86.8.7750

Walls, H. L., Wolfe, R., Haby, M. M., Magliano, D. J., de Courten, M., Reid, C. M., et al. (2010). Trends in BMI of urban Australian adults, 1980-2000. *Public Health Nutrition, 13*(5), 631–638. https://doi.org/10.1017/s1368980009991455

Westerterp, K. R., & Speakman, J. R. (2008). Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. *International Journal of Obesity, 32*(8), 1256–1263. https://doi.org/10.1038/ijo.2008.74

WHO. (2000). *Obesity: Preventing and managing the global epidemic (WHO technical report series 894)*. Geneva.

Wikarek, T., Chudek, J., Owczarek, A., & Olszanecka-Glinianowicz, M. (2014). Effect of dietary macronutrients on postprandial incretin hormone release and satiety in obese and normal-weight women. *British Journal of Nutrition, 111*(2), 236–246. https://doi.org/10.1017/S0007114513002389

Zwirska-Korczala, K., Konturek, S. J., Sodowski, M., Wylezol, M., Kuka, D., Sowa, P., et al. (2007). Basal and postprandial plasma levels of PYY, ghrelin, cholecystokinin, gastrin and insulin in women with moderate and morbid obesity and metabolic syndrome. *Journal of Physiology & Pharmacology, 58*(Suppl 1), 13–35.