Successful weight loss maintenance includes long-term increased meal responses of GLP-1 and PYY₃₋₃₆

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Abstract

Objective: The hormones glucagon-like peptide 1 (GLP-1), peptide YY₃₋₃₆ (PYY₃₋₃₆), ghrelin, glucose-dependent insulinotropic polypeptide (GIP) and glucagon have all been implicated in the pathogenesis of obesity. However, it is unknown whether they exhibit adaptive changes with respect to postprandial secretion to a sustained weight loss.

Design: The study was designed as a longitudinal prospective intervention study with data obtained at baseline, after 8 weeks of weight loss and 1 year after weight loss.

Methods: Twenty healthy obese individuals obtained a 13% weight loss by adhering to an 8-week very low-calorie diet (800 kcal/day). After weight loss, participants entered a 52-week weight maintenance protocol. Plasma levels of GLP-1, PYY₃₋₃₆, ghrelin, GIP and glucagon during a 600-kcal meal were measured before weight loss, after weight loss and after 1 year of weight maintenance. Area under the curve (AUC) was calculated as total AUC (tAUC) and incremental AUC (iAUC).

Results: Weight loss was successfully maintained for 52 weeks. iAUC for GLP-1 increased by 44% after weight loss ($P < 0.04$) and increased to 72% at week 52 ($P = 0.0001$). iAUC for PYY₃₋₃₆ increased by 74% after weight loss ($P < 0.0001$) and by 36% at week 52 ($P = 0.02$). tAUC for ghrelin increased by 23% after weight loss ($P < 0.0001$), but at week 52, the increase was reduced to 16% compared with before weight loss ($P = 0.005$). iAUC for GIP increased by 36% after weight loss ($P = 0.001$), but returned to before weight loss levels at week 52. Glucagon levels were unaffected by weight loss.

Conclusions: Meal responses of GLP-1 and PYY₃₋₃₆ remained increased 1 year after weight maintenance, whereas ghrelin and GIP reverted toward before-weight loss values. Thus, an increase in appetite inhibitory mechanisms and a partly decrease in appetite-stimulating mechanisms appear to contribute to successful long-term weight loss maintenance.

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Introduction

Weight regain after weight loss represents an unsolved challenge (1, 2). Thus, identification of hormonal changes associated with maintained weight loss is important as they may support or counteract successful long-term weight loss maintenance. The gut-derived incretin hormone, glucagon-like peptide 1 (GLP-1), may – together with peptide YY₃₋₃₆ (PYY₃₋₃₆) – be a key mediator

in postmeal satiety and thus the limitation of energy intake (3). The effect involves both peripheral and central mechanisms (4, 5). The postprandial GLP-1 response is attenuated in obese individuals (6, 7, 8), and recent studies have indicated that the impairment may be an early feature of obesity, as low GLP-1 responses are seen in overweight people independent of glucose tolerance

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status (9). In obesity, an inverse relationship between postprandial GLP-1 and insulin levels has been proposed, suggesting that insulin resistance may promote further weight gain through an impaired GLP-1 response (7). The observations regarding the effect of weight reduction on meal-induced GLP-1 responses in obesity are mainly derived from studies of obese subjects investigated after bariatric surgery, showing that postprandial GLP-1 levels are higher after Roux-en-Y gastric bypass (RYGB) than after laparoscopic assisted gastric bypass (10, 11). This observation may in part explain the higher degree of weight loss in RYGB compared with adjustable gastric banding patients (12). The secretory GLP-1 response to a meal after long-term weight reduction (up to a year) is unknown, and only few studies have examined the acute effect of diet-induced weight loss on basal and postprandial GLP-1 levels. Verdich *et al.* demonstrated an attenuated postprandial GLP-1 response in obese subjects, which was significantly increased after a 6-month 15% diet-induced weight loss and appeared to be intermediate between the GLP-1 responses for obese and lean subjects, pointing toward a gradual normalization of GLP-1 responses with weight loss (7). However, other weight loss intervention studies have reported decreased (13, 14, 15) and unaltered (16) postprandial GLP-1 levels after a diet-induced weight loss.

PYY₃₋₃₆ is another gut-derived satiety hormone, and postprandial levels are attenuated in obesity (17), perhaps in agreement with the finding that it is secreted together with GLP-1 from the intestinal L cells (18). The effect of weight loss on PYY₃₋₃₆ levels is, however, less clear, and both decreased (15) and unchanged (19) levels have been reported. Both fasting (20) and postprandial levels (21) increase after gastric bypass, and again, the effect seems to be larger after RYGB than after gastric banding.

The incretin hormone, glucose-dependent insulinotropic polypeptide (GIP), is secreted from the upper small intestine (22). In mice, GIP has been shown to promote energy storage via direct actions on adipose tissue by enhancing the activity of lipoprotein lipase (LPL) and to promote insulin-dependent free fatty acid uptake in adipocytes (23). Furthermore, a study has shown that GIP receptor knockout mice have reduced subcutaneous adipose tissue, improved insulin sensitivity, and were resistant to diet-induced obesity (24). However, a recent study examining 1405 individuals at low to high risk of developing type 2 diabetes found that high fasting GIP levels were associated with lower LDL levels, independent of insulin, indicating that high GIP levels may promote lipid clearance from the

blood (25). GIP probably does not possess any appetite-regulating properties, but levels have been suggested to be exaggerated in obesity and diabetes (26). The effect of weight loss on GIP levels is not well established in obesity, and both lower (7) and higher postprandial levels (27) have been reported.

The orexigenic hormone, ghrelin, is primarily secreted from the stomach and duodenum (28), and responds rapidly to a negative energy balance. Thus, after diet-induced weight loss, fasting ghrelin concentrations and, in parallel, feelings of hunger, increase (29). Therefore, ghrelin has been suggested to be involved in both short-term (meal initiation and termination) and long-term regulation of appetite and body weight (30).

Finally, the pancreatic hormones, glucagon and insulin, have also been suggested to be involved in satiety (31). Early studies have shown that administration of glucagon may reduce appetite in humans (32), but the physiological relevance of these findings has not been confirmed. However, combined administration of low doses of GLP-1 and glucagon inhibited food intake significantly and induced *c-fos* expression in the area postrema and amygdala in mice (33), and co-infusion of GLP-1 and glucagon has been shown to reduce food intake in humans (34).

Thus, the responses of appetite-regulating hormones after diet-induced weight loss remain unclear, and most studies have only assessed short-term effects of weight reduction. Therefore, the aim of this study was to assess meal-induced responses of the gastrointestinal hormones: GLP-1, GIP, PYY₃₋₃₆, ghrelin as well as the pancreatic hormones glucagon and insulin, both acutely and after 1 year of diet-induced weight loss maintenance in order to identify hormonal changes associated with maintained weight loss.

Subjects and methods

Twenty obese non-type 2 diabetic individuals (BMI: 30.0–39.9 kg/m²) aged 18–65 years were recruited for the study. Participants suffering from acute or chronic illnesses (including diabetes) or participants taking any form of medical treatment with known effects on glucose and lipid metabolism were excluded before entering the study. See previously published article (35) for a detailed study description.

Weight loss phase

The study participants were instructed by a clinical dietician on how to adhere to a low-calorie powder diet

of 810 kcal/day for 8 weeks. Products were provided by the Cambridge Diet (Cambridge Weight Plan, Corby, UK) (36). The low-calorie diet program consisted of a powdered formula mixture dissolved in skimmed milk or water. The program met all recommendations for daily intake of essential amino acids, fatty acids, vitamins, and minerals. During the weight loss phase, the participants had weekly consultations with the dietician who assisted them in achieving a body weight loss above 7.5%, which was required in order to continue into the 52-week weight loss maintenance program.

Weight loss maintenance phase

After 8 weeks on the low-calorie diet, the study participants followed Cambridge Weight Loss Maintenance Program with Cambridge Weight Plan products. Study participants were instructed to restrict calorie intake after calculation of their estimated daily energy need (from the Schofield equation (37) multiplied with the individual physical activity level score) and subsequently subtracted by 600 kcal (mean daily energy requirement subtracted by 600 kcal was 1395 ± 31 kcal, 95% CI (1267 – 1893). During the 52 weeks, the participants attended 13 consultations with the clinical dietician in which they received education on diet and lifestyle changes and were encouraged to follow the 'Nordic Nutrition Recommendations' (38). The participants kept track on their energy intake by recording this in a diet calendar which they brought to the consultations. In case of weight gain, up to two meals a day during the weight loss maintenance period were allowed to be replaced by Cambridge Weight Plan products to ensure a stable maintenance of weight loss.

Meal test

The study participants completed a 3-h meal test before weight loss, after weight loss, and after 52 weeks of weight loss maintenance. After an overnight fast (10–12 h), participants were placed resting in a recumbent position, and a cannula was inserted into a cubital vein for blood sampling. After fasting blood samples were drawn, the participants ingested an energy-dense nutrient drink (Fresubin; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) of 600 kilo calories (20% protein, 30% fat, and 50% carbohydrate). The meal was consumed over a period of 15 min. For each meal test, venous blood

samples were drawn 10, 5, and 0 min before (fasting state) and 15, 30, 45, 60, 90, 120, and 180 min after meal intake.

Outcome measures

Plasma PYY₃₋₃₆ and total plasma ghrelin were measured by radioimmunoassay (Millipore). All quality controls were within the prespecified limits. Radioimmunological determinations of total plasma GLP-1, total plasma GIP (total=sum of the active hormone and the primary metabolites, GLP-1 (9-36 amide), and GIP (3-42), respectively), and plasma glucagon were performed as described (39, 40, 41). The assays have a detection limit of less than 2 pmol/L and an intra-assay and inter-assay coefficients of variation of less than 6 and 15% respectively. Plasma glucose was measured with the glucose oxidase technique (YSI 2300 STAT Plus; Yellow Springs Instrument, Yellow Springs, OH, USA). Serum insulin concentrations were measured using Immulite 2000 solid-phase chemiluminescent immunometric assays (Immulite 2000; Siemens). Body composition (fat and lean body mass) was assessed using dual-energy X-ray absorptiometry scanning (Hologic discovery A, Bedford, MA, USA).

Ethical issues

The study was approved by the ethical committee in Copenhagen (reference number: H-4-2010-134) and was performed in accordance with the Helsinki Declaration II and with The International Council for Harmonisation of Good Clinical Practice (ICH-GCP). Participation in the investigation was voluntary, and the individuals could at any time retract their consent to participate (ClinicalTrials.gov Identifier: NCT02094183).

Statistical analysis

Within-subject variances from before to after weight loss and from before weight loss to week 52 were analyzed with paired *t*-tests, and linear regression analyses were made with log-transformed values (SPSS statistics version 22; IBM). Total and incremental areas under the curve (AUC) were calculated using Prism Version 5.04 (GraphPad) by the trapezoidal method, which is a validated tool for postprandial assessments (42). Incremental AUC (iAUC) was defined as the area above the baseline (defined by mean hormone levels at *t*=−15, −5, and 0 min before meal ingestion), thus covering time points 0–180 min.

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Incremental AUC was used when there were no differences in fasting (from baseline to week 52) whereas total AUC was used when there were differences in fasting levels in order to account for relevant hormone response.

Homeostasis model assessment (HOMA) for insulin sensitivity was calculated as follows (43):

$$\text{HOMA-IR} = \frac{(\text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{IU/mL)})}{22.5}$$

The Matsuda index of insulin sensitivity was calculated as follows (44):

$$\text{Matsuda index} = \frac{10.000}{\sqrt{(\text{fasting insulin} \times \text{fasting glucose} \times 2\text{-h insulin} \times 2\text{-h glucose})}}$$

The Matsuda index was calculated with insulin in $\mu\text{U/mL}$ and glucose in mg/dL . Data were obtained from meal tests as described previously rather than from an oral glucose tolerance test. Data are shown as mean $\pm$ S.E.M. A two-tailed *P*-value less than 0.05 was considered significant.

Data were tested for normal distribution with the Shapiro–Wilk test.

Results

Weight change and body mass composition

After the very-low calorie diet, the study participants achieved a total weight loss of 12.5 kg equivalent to 13% of initial body weight, $P < 0.0001$. After 1 year, the study participants had maintained their weight loss, with no significant weight difference from after initial weight loss to week 52 (weight difference: 2.2 kg (95% CI: -1.2 to 5.6), $P = 0.2$). Fat mass decreased by 6.3% from before to after weight loss ($P < 0.0001$), and by week 52, the total fat mass decrease was 8.3% ($P = 0.002$) compared with before weight loss. Lean body mass (calculated without bone mass) increased by 3.8% from before to after weight loss ($P < 0.0001$), and after 52 weeks of weight loss maintenance, the lean body mass increased additionally by 1.4% to a total of 5.2% ($P < 0.0001$) from before weight loss (Table 1).

Hormonal changes

The incremental area under the GLP-1 response curve increased by 44% ($P = 0.04$) from before to after weight loss and increased by additional 28% at week 52, that is,

a total increase of 72% compared with before weight loss ($P = 0.0001$). Fasting GLP-1 levels remained unchanged (Fig. 1A).

Incremental area under the GIP response curve increased by 36% ($P = 0.001$) from before to after weight loss, but at week 52, GIP levels had returned to before-weight loss values ($P = 0.9$). Fasting GIP levels remained unchanged (Fig. 1B).

The incremental area under the PYY₃₋₃₆ response curve increased by 74% ($P < 0.0001$) from before to after weight loss, and by week 52, the incremental area under the curve was increased by 36% from before-weight loss values ($P = 0.02$). Fasting plasma levels of PYY₃₋₃₆ decreased by 13% from before to after weight loss ($P = 0.03$) but reverted to before-weight loss values at week 52 ($P > 0.05$) (Fig. 1C).

Total area under the ghrelin response curve increased by 23% from before to after weight loss ($P < 0.0001$), but by week 52, the total increase from before weight loss was reduced by 7% to a total of 16% ($P = 0.005$). Fasting ghrelin levels increased by 25% from before to after weight loss ($P < 0.0001$), but at week 52, the increase was reduced to 17% from before weight loss ($P = 0.01$) (Fig. 1D). Meal-stimulated as well as fasting glucagon levels remained unchanged throughout the study period (Fig. 1E).

Total area under the insulin response curve decreased by 19% from before to after weight loss ($P = 0.02$) and remained significantly lower throughout the weight loss maintenance period (17% lower than before weight loss, $P = 0.04$). Fasting insulin levels decreased by almost 40% immediately after weight loss ($P < 0.0001$) and remained low throughout the weight loss maintenance phase, although tended to revert toward before-weight loss values (17% lower than before weight loss, $P = 0.002$) (Fig. 1F).

HOMA-IR decreased significantly from before weight loss to after weight loss (estimated difference: -0.96 (95% CI: 0.5 – 1.4), $P < 0.0001$) and remained lower than before weight loss after 52 weeks (estimated difference: -0.4 (95% CI: 0.1 – 0.7), $P = 0.01$) (Table 2).

The Matsuda index of insulin sensitivity improved after weight loss (estimated difference: 0.5 (95% CI: 0.1 – 0.9), $P = 0.016$ and tended to remain elevated at week 52, although not significantly (estimated difference: 0.4 (95% CI: 0.04 – 0.8), $P = 0.08$) (Table 2).

Discussion

In this study, we were able to demonstrate that after 1 year of successful 13% body weight loss maintenance, the meal responses of the appetite-inhibiting hormones

Table 1 Subject characteristics (Age = 43.4 ± 9.6; n = 20) at screening, baseline, week 52, and differences between means from screening to baseline and week 52. Absolute values are shown as ± s.e.m. Differences between means are shown with 95% CIs.

	Screening	Baseline	Week 52	Difference between means from	
				Screening to baseline	Screening to week 52
Weight (kg)	95.2 ± 8.1	82.8 ± 7.6	85.0 ± 10.6	-12.5 (-11.2 to -13.7)***	-10.2 (-6.4 to -14.0)***
BMI (kg/m ²)	33.5 ± 2.2	29.1 ± 1.9	29.8 ± 3.2	-4.4 (-3.8 to -4.9)***	-3.7 (-1.9 to -5.5)***
Fat mass (%)	42.1 ± 1.1	39.5 ± 1.2	38.5 ± 1.3	-2.64 (-3.3 to -1.9)***	-3.5 (-5.4 to -1.5)**
Lean mass (%)	55.3 ± 0.7	57.5 ± 0.9	58.6 ± 1.0	2.1 (1.5-2.7)***	2.9 (1.9-3.9)***
GLP-1 (180 min × pmol/L)					
Total AUC	2423 ± 205	3103 ± 261	3287 ± 221	680 (231-1129)**	864 (542-1186)***
Incremental AUC	1108 ± 162	1595 ± 182	1911 ± 135	488 (12-962)*	803 (405-1200)***
Fasting concentration (pmol/L)	7.5 ± 4.9	8.1 ± 3.3	7.4 ± 3.4	0.6 (-2.5 to 1.3) NS	-0.1 (-1.4 to 1.2) NS
PYY ₃₋₃₆ (180 min × pg/mL)					
Total AUC	15783 ± 939	17214 ± 1144	17786 ± 1063	1431 (299-3161) NS	2306 (1281-3331)***
Incremental AUC	3315 ± 464	5786 ± 554	4465 ± 512	2470 (1256-3685)***	1188 (191-2185)*
Fasting concentration (pg/mL)	73.9 ± 6.1	63.7 ± 5.5	77.6 ± 7.5	-10.1 (-19.1 to 1.2)*	6.2 (5.9-18.5) NS
Ghrelin (180 min × pg/mL)					
Total AUC	129168 ± 12904	158899 ± 16039	150382 ± 16646	29732 (16938-42525)***	21214 (35019-7409)**
Decremental AUC	35572 ± 6681	46280 ± 5048	45967 ± 5625	10708 (-814-2231) NS	10395 (2478-18312)*
Fasting concentration (pg/mL)	926.1 ± 113.4	1154.5 ± 110.5	1083.7 ± 127.7	228.4 (120.4-336.4)***	157.8 (35.9-279.9)**
GIP (180 min × pmol/L)					
Total AUC	9972 ± 743	12807 ± 1137	9918 ± 666	2835 (993-4676)**	-54.1 (-1175-1067) NS
Incremental AUC	8589 ± 728	11684 ± 1074	8609 ± 623	3096 (1397-4794)**	21 (1066-1107) NS
Fasting concentration (pmol/L)	7.3 ± 0.9	10.7 ± 3.4	10.9 ± 3.5	3.3 (-4.5-11.1) NS	3.7 (-3.6 to 10.9) NS

*P < 0.05, **P < 0.01, ***P < 0.0001. NS, Not significant.

GLP-1 and PYY₃₋₃₆ remain significantly increased, whereas the orexigenic hormone ghrelin and the lipid-regulating hormone GIP after an initial increase revert toward before-weight loss values. Glucagon levels were not affected by weight loss.

In 1997, Näslund *et al.* described elevated levels of both fasting and postprandial GLP-1, PYY₃₋₃₆, and cholecystokinin (CCK) 20 years after jejunoileal bypass surgery. However, it was unsettled whether this increase was associated with the weight loss or the operation, which in itself induced a change in gut hormone secretion due to increased exposure of the terminal ileum to food components (45). Today, it is well recognized that postprandial GLP-1 and PYY₃₋₃₆ secretion is increased in obese patients after bypass surgery (20). Thus, in patients receiving RYGB surgery, the increase in GLP-1 and PYY₃₋₃₆ is evident before significant weight loss and is thought to be due to the altered transit of nutrients through the gut (46). However, it is of notice that in studies of good and poor RYGB responders (with larger and smaller weight losses, respectively), PYY₃₋₃₆ and GLP-1 responses were associated with the magnitude of the weight loss (47, 48). Additionally, it was shown that suppression of GLP-1 and PYY₃₋₃₆ with somatostatin was associated with the return of appetite and food intake (48), and it was suggested that poor weight loss maintenance might be associated with attenuated postprandial responses of PYY₃₋₃₆ and GLP-1 compared with patients with sustained weight losses (47, 48). Thus, these studies might suggest that the ability of the patients to secrete the appetite inhibitory hormones would predict the resulting weight loss.

Based on the current results, we propose that the ability to maintain weight loss depends on the restoration of the secretion of the appetite-regulating hormones. Furthermore, weight gain may be associated with a gradual impairment in the secretion of these hormones, initiating a vicious cycle with progressively deteriorating appetite regulation. The changes we observe do not seem compatible with decreased secretion of the appetite-regulating hormones as a primary cause of obesity, because a primary deficiency would not be expected to disappear after weight loss. Thus, the increase in GLP-1 and PYY₃₋₃₆ responses after long-term weight loss supports the concept of decreased hormone responses in obesity and therefore secondary to the obese state. Accordingly, lifting of obesity (and especially reducing fat mass) ameliorates impairment of secretion and facilitates maintenance of a weight loss.

We observe a significant improvement in both HOMA-IR and the Matsuda index of insulin sensitivity

Figure 1

Plasma hormone responses to a meal test before weight loss (circle full line), after weight loss (triangle dotted line), and after 1 year of weight loss maintenance (square stippled line). (A) Area under the GLP-1 response curve. (B) Area under the GIP response curve. (C) Area under the PYY₃₋₃₆ response. (D) Area under the ghrelin response curve. (E) Area under the glucagon response curve. (F) Area under the insulin response. Liquid meals were ingested at timepoint 0.

immediately after weight loss, and HOMA-IR remained low after 1 year of weight loss maintenance. This supports the hypothesis that the GLP-1-secreting L cells exhibit insulin sensitivity and thus develop some degree of insulin resistance in the obese state (49). Accordingly, the observed improvement in insulin sensitivity after acute and maintained fat mass loss could explain the sustained increase in GLP-1 release, supporting the notion of an inverse correlation between GLP-1 and insulin (7). PYY₃₋₃₆ secretion showed similar increases along with changes in fasting basal secretion, making the pattern less clear. However, it is now known that not all L cells secrete both PYY₃₋₃₆ and GLP-1 – many particularly proximal L cells secrete only GLP-1 – and this may explain the discrepant findings of weight loss effects on fasting PYY₃₋₃₆ and GLP-1 levels (50).

It is well known that obese individuals display lower levels of the hunger hormone ghrelin (51) and that concentrations rapidly increase during dietary restriction and exercise (29, 52). Furthermore, it has been suggested that ghrelin plays a role in long-term weight maintenance (30). Our results demonstrate that ghrelin levels respond rapidly to acute energy restriction, but gradually return to before-weight loss values after 1 year of weight loss maintenance, indicating that ghrelin is a

short-term regulator of appetite. Furthermore, that the acute ghrelin-mediated appetite stimulation that occurs during acute weight loss is transient and that long-term adaptation to a new weight level does not necessarily involve increased ghrelin-mediated appetite stimulation.

The early increased ghrelin levels might be viewed as a compensatory mechanism to preserve body weight even if excessive. However, after long-term weight loss maintenance, ghrelin concentrations have almost returned to before-weight loss levels, which may be viewed as an adaptation response and actually helps to facilitate maintenance of the new body weight especially combined with the sustained increase in the appetite hormones GLP-1 and PYY₃₋₃₆.

We report a temporary increase in the intestinal hormone GIP in acute response to weight loss. Our results are in contrast to those of Verdich *et al.*, who reported an impaired postprandial GIP response after weight loss (7), leading to a hypothesis of exaggerated levels in obesity, which become normalized when excess body weight is lost (26). Our findings are in agreement with the observations by Raben *et al.*, who reported an increase in postprandial GIP levels in

Table 2 Subject characteristics (age = 43.4 ± 9.6 ; $n = 20$) at screening, baseline, week 52, and differences between means from screening to baseline and to week 52. Absolute values are shown as $\pm$ s.e.m. Differences between means are shown with 95% CIs.

	Screening	Baseline	Week 52	Difference between means from	
				Screening to baseline	Screening to week 52
Glucagon (180 min $\times$ pg/mL)					
Total AUC	868.2 ± 78.4	839.6 ± 61.9	792.5 ± 72.6	-28.6 (-157.7 to 100.5) NS	-75.7 (-211.9 to 60.5) NS
Incremental AUC	214.4 ± 23.1	168.9 ± 16.5	180.6 ± 19.1	-45.4 (-98.6 to 7.8) NS	-33.8 (-85.4 to 17.8) NS
Fasting concentration (pg/mL)	4.2 ± 0.5	4.5 ± 0.4	3.8 ± 0.4	0.3 (-0.46 to 1.1) NS	-0.3 (-1.2 to 0.5) NS
Insulin (180 min $\times$ pmol/L)					
Total AUC	64099 ± 6140	51657 ± 3769	52301 ± 3966	-12443 (-22761 to -2125)*	-11090 (-21810 to -370)*
Incremental AUC	50177 ± 5493	43799 ± 3460	41770 ± 3273	-6377 (-15962 to 3207) NS	-7808 (-18110 to 2495) NS
Fasting concentration (pmol/L)	68.3 ± 6.9	41.5 ± 3.6	56.4 ± 5.6	-26.8 (-36.6 to -17.1)*	11.9 (-18.8 to -4.9)*
Glucose (180 min $\times$ mmol/L)					
Total AUC	1060 ± 33	1114 ± 27	1057 ± 35	54 (-5 to 113) NS	-3 (-68 to 62) NS
Incremental AUC	156 ± 15	212 ± 20	158 ± 19	57 (14 – 100)*	2 (-38 to 43) NS
Fasting concentration (mmol/L)	5.0 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	-0.2 (-0.5 to 0.05) NS	0.03 (-0.22 to 0.3) NS
HOMA-IR	2.3 ± 1.3	1.3 ± 0.6	1.8 ± 0.9	-0.96 (0.5 – 1.4)*	-0.4 (0.1 – 0.7)*
Matsuda	1.8 ± 0.7	2.7 ± 1.3	2.3 ± 0.8	0.54 (0.11 – 0.97)*	0.38 (0.05 – 0.8)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$. FPG, fasting plasma glucose; FINS, fasting insulin; 2HINS, 2-h insulin; 2HPG, 2-h plasma glucose; NS, Not significant.

post-obese women compared with obese controls (27). The obesogenic effect of postprandial GIP is well described and is probably attributed to the anabolic effect of the hormone on adipose tissue rather than a centrally mediated effect on appetite regulation (53). Although not verified, it could be speculated that, as for ghrelin, the acute increases in postprandial GIP following weight loss are part of the body's acute compensatory mechanisms to avoid depletion of energy stores, and increasing postprandial GIP levels represent an attempt to facilitate fat storage. However, as for ghrelin, the response is only temporary – when weight loss is sustained, postprandial GIP returns to before-weight loss levels, thereby, with time, facilitating weight maintenance.

Glucagon levels did not change throughout the study period, and besides a few early studies (32, 54), no documentation for a direct appetite-regulating effect in humans has been demonstrated. Thus, we conclude that glucagon does not seem to be involved in weight loss mechanisms in humans, although it may act synergistically in combination with GLP-1 (34).

Finally, a surprising finding in the study was an increase in lean body mass during the weight loss program. However, the composition of the diet (more protein) combined with a possibly increased level of exercise may have led to an increase in muscle mass and therefore lean body mass.

Strengths and limitations

We were able to introduce and maintain a large fat mass loss over 1 year, which made it possible to analyze any accompanying changes in hormonal response during maintained fat loss uninfluenced by fat regain. We performed meal tests in this study, which is the golden standard for evaluating GLP-1, PYY₃₋₃₆, ghrelin, and GIP responses, and also provides information about glucagon secretion. We used well-documented assay methods with high specificity and sensitivity for analyzing the plasma samples (39, 40, 41). Many studies of hormone secretion have been carried out with various commercially available kits, although the specificity and sensitivity of these kits for hormonal analysis may vary considerably (55). For instance, measurements of peripheral circulating levels of the intact GLP-1 molecular form, GLP-1-(7–36)NH₂/GLP-1-(7–37), only reflect the small fraction of the hormone that reaches its targets via the classical endocrine route after having exerted the predominant part of its actions via the

nervous system (56); such assays therefore are not suitable for measurements of GLP-1 secretion, which requires determination of the sum of the intact form and its primary, N-terminally truncated metabolites. Finally, we have measured total ghrelin that comprises both the acylated and the deacylated forms and thus represents total ghrelin secretion (57, 58).

Conclusions

Collectively, we show that after 1 year of weight loss maintenance, meal responses of the appetite-inhibiting hormones GLP-1 and PYY₃₋₃₆ remain significantly increased, whereas levels of the orexigenic hormone ghrelin and the lipid-regulatory hormone GIP tend to revert toward before-weight loss values, indicating that an increase in appetite-inhibiting mechanisms and a partly decrease in appetite-stimulating mechanisms appear to contribute to successful long-term weight loss maintenance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S S T, J J H, and S M designed the study. E W I and J L conducted the study and collected data. E W I wrote the manuscript and analyzed data. J J H, S M, S S T and L H contributed to discussion, reviewed/edited the manuscript and approved the final version.

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