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Circulating levels of gastrointestinal hormones in response to the most common types of bariatric surgery and predictive value for weight loss over one year: Evidence from two independent trials

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ABSTRACT

Aims: Bariatric surgery leads to profound and sustainable weight loss. Gastrointestinal hormones are involved in energy and glucose homeostasis, thus postoperative changes of their circulating levels may be mediating future weight loss. To investigate how the circulating concentrations of gastrointestinal hormones change in response to the most common types of bariatric operation and whether these changes can predict future weight loss.
Materials and Methods: We measured circulating GLP-1, GLP-2, oxyntomodulin, glicentin, glucagon, major proglucagon fragment (MPGF), ghrelin, GIP, PYY after overnight fasting and/or after a mixed meal test (MMT) in: a) 14 subjects that have undergone either an adjustable gastric banding [AGB] (n = 9) or a Roux-en-Y bypass (RYGB) (n = 5) (Pilot study 1), b) 28 subjects that have undergone either a vertical sleeve gastrectomy (n = 17) or a RYGB (n = 11) before and three, six and twelve months after surgery.
Results: In addition to the expected associations with GLP-1, the most robust increases were observed in postprandial levels of oxyntomodulin and glicentin three months after VSG or RYGB (but not after AGB) and are associated with degree of weight loss. Oxyntomodulin and glicentin levels at the third and sixth month postoperative visit are positively associated with feeling of satiety which may be underlying the observed associations with future weight loss.
Conclusion: Beyond GLP-1, early postprandial changes in circulating oxyntomodulin and glicentin are predictors of weight loss after bariatric surgery, possibly through regulation of satiety. Further studies should focus on underlying mechanisms, and their potential as attractive therapeutic tools against obesity and related comorbidities.

1. Introduction

Bariatric surgery leads to major metabolic changes characterized by two hallmarks: (a) rapid improvement of glucose homeostasis and even resolution of type 2 diabetes (T2D) shortly after operation and largely independently of weight loss [1–3], (b) profound and sustainable long-term weight loss mainly due to reduction of appetite regulation and energy intake [1–3]. Several studies have demonstrated that the circulating profile of gastrointestinal (GI) hormones, especially postprandially, changes robustly after bariatric operation and to date GLP-1 seems to be the most important regulator of glucose homeostasis in this condition [1–3]. These observations have supported the rationale for developing GLP-1-related treatments against obesity and its comorbidities such as T2D [4–6].

Beyond established hormone changes such as the above, no information is available about any associations of novel GI hormones with observed weight loss after bariatric surgery. Identifying these associations may help us develop therapeutic strategies to treat obesity and diabetes and will inform decisions on evaluation of combination therapies including more than one GI hormones (e.g. GLP-1 with GIP or with glucagon) [4–6].

The aims of this study were i) to perform a comparative analysis of circulating levels of nine, traditional and novel, gastrointestinal

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hormones at the fasting state as well as during a mixed-meal test in morbidly obese patients before and after undergoing one of the three most popular bariatric interventions i.e. laparoscopic adjustable gastric banding [AGB], Roux-en-Y gastric bypass [RYGB] or vertical sleeve gastrectomy [VSG], ii) to identify which GI hormone changes are most closely related to weight changes over time and whether GI hormone changes at earlier time points may predict future weight loss, and iii) to investigate whether any GI hormone changes may also be associated with increased satiety as well as with any improvements in components of the metabolic syndrome as potential predictors of future comorbidities. The GI hormones we studied were: a) all six hormones deriving from the posttranslational processing of proglucagon peptide, among them four secreted primarily from the gut i.e. glucagon like peptide-1 (GLP-1), glucagon like peptide-2 (GLP-2), oxyntomodulin and glicentin, and two secreted mainly from the pancreas, i.e. glucagon and Major Proglucagon Fragment (MPGF) [7], b) the incretin gastric inhibitory peptide (GIP) secreted by the duodenum and jejunum, c) the appetite-regulating hormone ghrelin secreted from the stomach, d) the ileum and colon-secreted peptide-YY (PYY).

2. Materials and Methods

2.1. Study 1 - Pilot

This first pilot study included fourteen morbidly obese subjects recruited and followed from 2010 to 2013 at Beth Israel Deaconess Medical Center (BIDMC) (s. Flow Diagram Study 1, Supplemental material p. 9). The protocol has been partially described [8]. The subjects underwent either a RYGB (n = 5) or an AGB (n = 9). The type of surgery was selected after clinical evaluation. Inclusion and exclusion criteria are described in Supplemental appendix (s. Supplemental material, page 1). Subjects were examined prior to surgery and at 2, 3, 6 and 12 months after operation in the BIDMC General Clinical Research Center (GCRC) and underwent anthropometric measurements, body composition evaluations, and morning blood draws after overnight fasting. Blood samples were available for these subjects at 3 and 6 months after surgery. Five non-diabetic subjects completed a mixed nutrient stimulation study (mixed meal test, [MMT]) consisting of 53% of carbohydrates, 22% of fat and 25% of protein during the baseline and 6-month visit. Blood samples were collected every 30 min after meal ingestion for 2 h. No important adverse events related to the blood draws or MMTs were observed.

2.2. Study 2

The protocol of this study has been previously described [9]. From that study, serum was available from twenty-eight morbidly obese subjects, in most cases before and three, six and twelve months after operation (s. Flow Diagram Study 2 and Supplemental Table 1 in Supplemental material pages 10–11). These subjects underwent either a RYGB (n = 11) or a vertical sleeve gastrectomy (VSG, n = 17). The type of surgery was selected after clinical evaluation. Inclusion and exclusion criteria are described in Supplemental appendix (s. Supplemental material page 1). Subjects were examined prior to surgery and at 3, 6, and 12 months post operatively at the First Department of Propaedeutic Internal Medicine, Laiko General Hospital and underwent anthropometric measurements and body composition evaluations, as well as morning blood draws after overnight fasting (recruitment and follow-up 2006–2013). All subjects completed an MMT (consumption of 200 ml ice cream within 5 min), consisting of 59% of kcal from fat, 33% carbohydrates and 8% protein. Blood samples were collected prior to MMT and every 30 min postprandially for 3 h. No important adverse events related to the blood draws or MMTs were observed.

2.3. Anthropometric, Body Composition and Biochemical Measurements

Anthropometric and body composition measurements are described in Supplementary material page 3–4.

GLP-1, GLP-2, oxyntomodulin, MPGF and glucagon were measured with enzyme-linked immunosorbent assay (ELISA) obtained from Ansh Laboratories (Webster, TX, USA), glicentin from Mercodia (Uppsala, Sweden), GIP, PYY and ghrelin from Millipore (Bedford, MA, USA).

Assay characteristics including cross-reactivity are described in Supplemental material (s. pages 5–8).

2.4. Statistics

Statistical analysis was performed with SPSS v19.0 (SPSS, Inc., Chicago, IL) for Windows and with GraphPad Prism 7 (GraphPad Software Inc., La Jolla, CA) and is described in Supplemental material page 2–3.

2.5. Study Approval and Trial Registration

Both studies were registered at clinicaltrials.gov (registration numbers: Study 1 - NCT03853590, Study 2 - NCT03851874). Study 1 was approved by the Institutional Review Board of BIDMC and Study 2 by the ethics committee of Laiko General Hospital, Athens, Greece. Written informed consent was obtained from all participants in both studies. Both studies were in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice.

3. Results

3.1. Circulating Profile of GI Hormones After AGB and/or RYGB in Pilot Study 1

Changes in baseline anthropometric and biochemical parameters of the 14 subjects participating in Study 1 are summarized in Supplemental Table 2 (p. 12 in Supplemental material). A significant weight loss (~18%) and fat mass loss (~28%) are observed 6 months after the operation, which are more profound in subjects undergoing RYGB compared to AGB.

The pre-prandial circulating concentrations of the GI hormones remain stable after bariatric operation, with the exception of a reduction in circulating GLP-1 and GLP-2 (s. Supplemental Table 3, p.13 in Supplemental material). This reduction was mainly observed in the subjects that underwent a RYGB. Five subjects (three undergoing an AGB and two undergoing a RYGB) had a MMT before and six months after operation (s. Supplemental Figs. 1 and 2, p. 16–17 in Supplemental material). Subjects undergoing an AGB had minor changes in the circulating levels of all the hormones. In contrast, both RYGB patients demonstrated a robust increase in GLP-1, glicentin, PYY and a decrease in MPGF postoperatively.

Collectively, the first pilot study demonstrated little to no changes in the circulating levels of the gastrointestinal hormones in patients that have undergone an AGB, whereas it provided indications for changes after RYGB. We then proceeded with evaluation of subjects undergoing *RYGB or VSG in study 2*.

3.2. Circulating Profile of GI Hormones After RYGB or VSG in Study 2

Based on the findings from the first study, we investigated the circulating profile of the study hormones in a larger number of subjects undergoing RYGB or VSG, who were followed for a longer period (up to one year). Although the subjects were not formally matched or randomized to the type of operation, at baseline (before operation) there was no difference in weight, fat mass, blood pressure, lipid profile or RMR between VSG and RYGB group (s. Supplemental Table 4, p. 14 in Supplemental material). Similar weight (30–32%) and fat loss (26–37%) were observed after RYGB and VSG. Both fasting (0 min in MMT) and postprandial (30–180 min in MMT) insulin and glucose were reduced post-operatively (s. Fig. 1a–b).

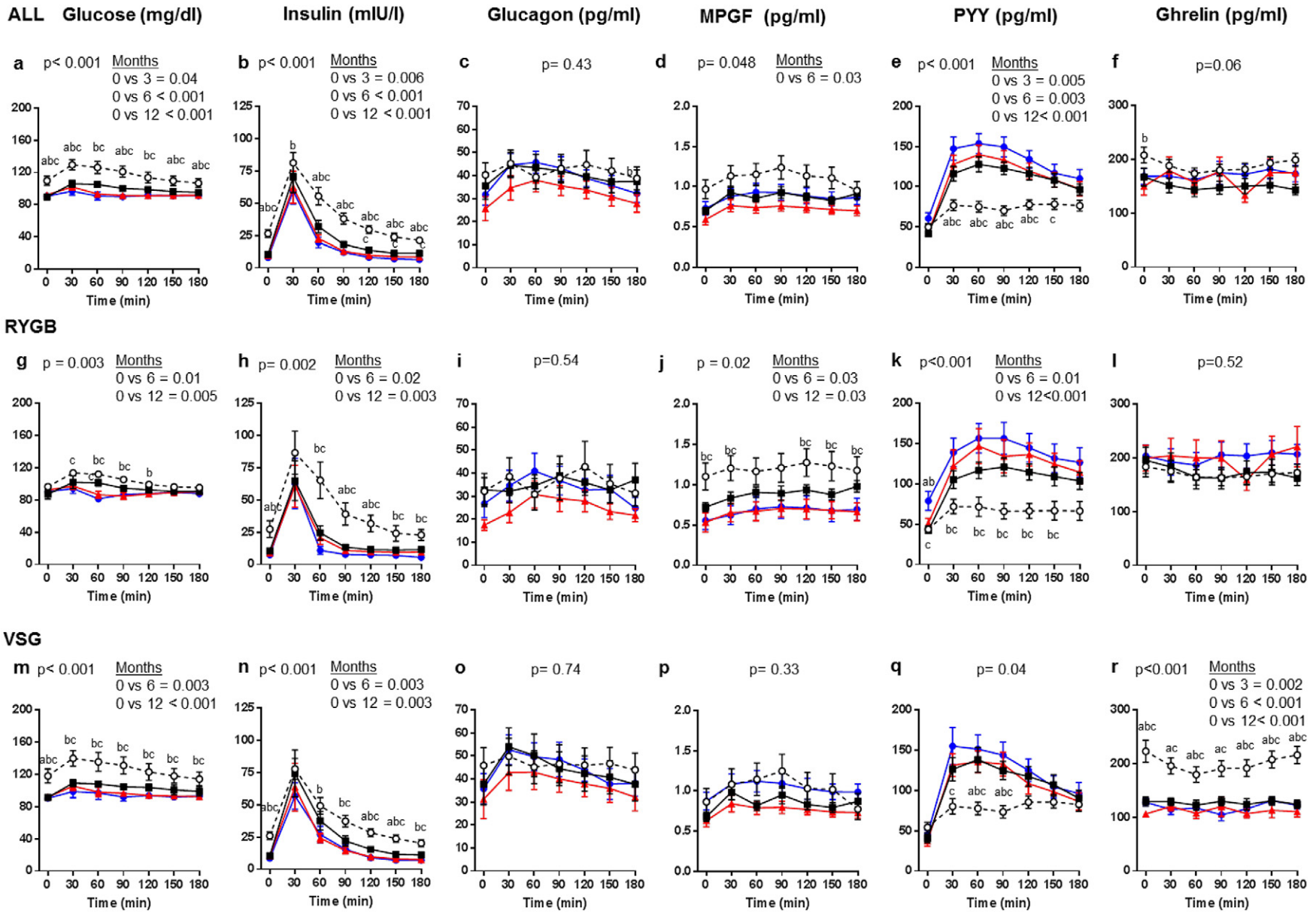

Fig. 1. Circulating profile of insulin, glucose, glucagon, MPGF, PYY and ghrelin in all subjects of study 2 (n = 28) (a–f) as well as in subjects receiving a RYGB (n = 11) (g–l) or a VSG (n = 17) (m–r) during a MMT. p refers to one-way ANOVA or the Kruskal-Wallis test between area under the curve (AUC) before and three, six and twelve months after operation. Values below "Months" indicate p-value in the post-hoc Tukey's or Dunn's test between the AUCs of the months. Letters a, b, c indicate p-value < 0.05 in the post-hoc Tukey's or Dunn's test between the different months for the specific time point of the MMT (these tests were performed only when one-way ANOVA or the Kruskal-Wallis test was <0.05), i.e. "a" shows p < 0.05 compared to values at 3 months, "b" compared to values at 6 months and "c" compared to values at 12 months. Lines: Dotted = Before operation, black = 3 months, red = 6 months, blue = 12 months after operation. Means ± SEM presented.

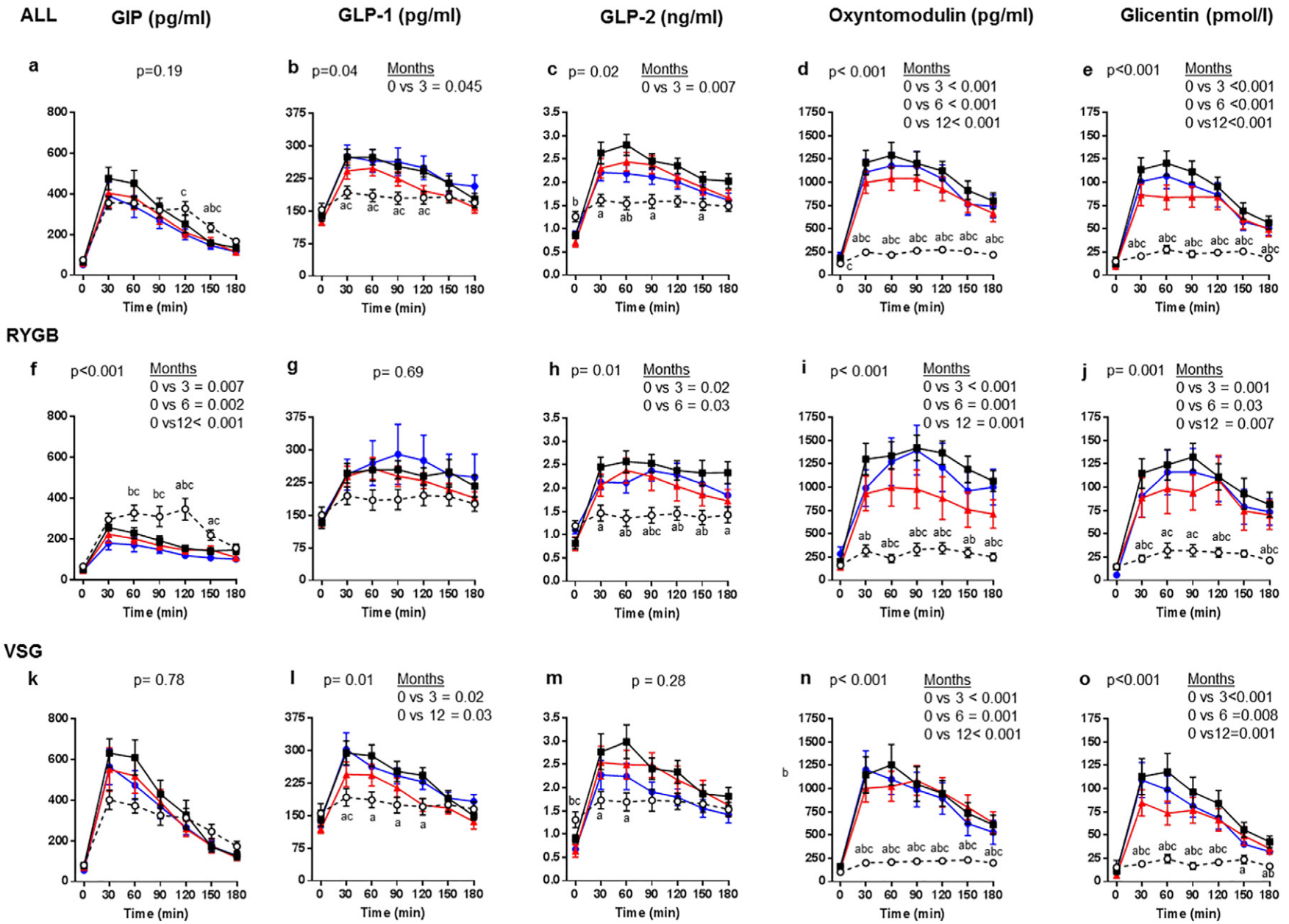

Fig. 2. Circulating profile of incretins, i.e. GIP, GLP-1, GLP-2, oxyntomodulin and glicentin in all subjects of study 2 (n = 28) (a–e) as well as in subjects receiving a RYGB (n = 11) (f–j) or a VSG (n = 17) (k–o) during a MMT. p refers to one-way ANOVA or the Kruskal-Wallis test between area under the curve (AUC) before and three, six and twelve months after operation. Values below "Months" indicate p-value in the post-hoc Tukey's or Dunn's test between the AUCs of the months. Letters a, b, c indicate p-value < 0.05 in the post-hoc Tukey's or Dunn's test between the different months for the specific time point of the MMT (these tests were performed only when one-way ANOVA or the Kruskal-Wallis test was <0.05), i.e. "a" shows p < 0.05 compared to values at 3 months, "b" compared to values at 6 months and "c" compared to values at 12 months. Lines: Dotted = Before operation, black = 3 months, red = 6 months, blue = 12 months after operation. Means ± SEM presented.

Pre-prandial concentrations (0 min) of PYY were higher twelve months after operation and of MPGF were lower six and twelve months after operation in the RYGB group (Fig. 1j–k). In the VSG group, GLP-2 levels were lower six and twelve months postoperatively (Fig. 2m), while ghrelin levels were profoundly lower after 3 and up to at least 12 months (Fig. 1r).

Postprandially, there was a profound increase in the AUC of oxyntomodulin and glicentin (Fig. 2d–e) and secondarily of GLP-1 (Fig. 2b), GLP-2 (Fig. 2c), PYY (1e) and a decrease in the AUC of MPGF (Fig. 2d). The changes in AUCs in all hormones were already established the first three months post-operatively and were largely maintained throughout the first twelve postoperative months.

The increase in AUCs of oxyntomodulin, glicentin and PYY were largely independent of the type of operation (s. Fig. 2i,j,n,0 and Fig. 1k, q). The increase in GLP-1 was more profound in the VSG group and of GLP-2 in the RYGB group (s. Fig. 2g,l and h–m). Reductions in the

Table 1
Relative AUC changes of nine GI hormones over the study time period and spearman correlation coefficients of them with the relative change of weight 3, 6 or 12 months after operation in Study 2.

Hormones (% change of AUC)	3 months after OP		6 months after OP		12 months after OP	
	Median (25th–75th)	Sig.	Median (25th–75th)	Sig.	Median (25th–75th)	Sig.
1. GIP (pg/ml)	−3.7 (−36.7, 65.8)	4,5	−13.4 (−36.5, 2.7)	4,5	−31.5 (−50.8, 4.1)	4,5,8
2. GLP-1 (pg/ml)	35.1 (−10.3, 130.4)	4,5	23.6 (−5.3, 74.1)		41.6 (4.8, 91.4)	
3. GLP-2 (ng/ml)	24.8 (−3.8, 126.8)	4,5	54.0 (−9.1, 123.5)		7.1 (−25.3, 80.9)	4,5
4. Oxm (pg/ml)	315.0 (143.1, 752.6)	1,2,3, 6,7,8,9	345.7 (108.7, 460.2)	1,6,7,9	307.6 (100.5, 533.3)	1,3,6,7,9
5. Glicentin (pmol/l)	289.7 (130.6, 832.4)	1,2,3, 6,7,8,9	280.0 (27.0, 501.1)	1,6,7,9	227.1 (120.6, 587.1)	1,3,6,7,9
6. Glucagon (pg/ml)	−14.6 (−33.9, 95.6)	4,5	−26.0 (−38.5, 60.1)	4,5	1.6 (−52.9, 170.9)	4,5
7. MPGF (ng/ml)	−14.0 (−37.1, 36.9)	4,5	−29.4 (−57.3, 1.0)	4,5	−21.5 (−46.8, 116.8)	4,5
8. PYY (pg/ml)	52.7 (−10.5, 105.9)	4,5	47.6 (−2.5, 119.1)		68.6 (15.4, 148.3)	1
9. Ghrelin (pg/ml)	−18.7 (−42.1, 10.1)	4,5	−16.0 (−44.6, 3.5)	4,5	−4.9 (−37.0, 26.7)	4,5
P-overall	<0.001		<0.001		<0.001	

Hormones (% change of AUC)	Weight (% change)					
	3 months after OP		6 months after OP		12 months after OP	
	R	*Pa*	*R*	*Pa*	*R*	*Pa*
3						
GIP (pg/ml)	0.071	0.76	−0.173	0.47	−0.053	0.84
GLP-1 (pg/ml)	−0.142	0.51	**−0.432**	**0.045**	**−0.405**	**0.077**
GLP-2 (ng/ml)	−0.074	0.74	0.096	0.69	0.025	0.91
Oxm (pg/ml)	−0.250	0.24	**−0.553**	**0.008**	**−0.504**	**0.024**
Glicentin (pmol/l)	**−0.365**	**0.079**	**−0.635**	**0.001**	**−0.618**	**0.004**
Glucagon (pg/ml)	0.111	0.61	0.069	0.76	−0.161	0.50
MPGF (ng/ml)	−0.258	0.25	**−0.505**	**0.019**	**−0.425**	**0.070**
PYY (pg/ml)	−0.224	0.28	−0.059	0.79	0.005	0.98
Ghrelin (pg/ml)	0.099	0.75	0.091	0.80	−0.383	0.31
6						
GIP (pg/ml)			−0.174	0.48	−0.356	0.18
GLP-1 (pg/ml)			−0.349	0.12	**−0.414**	**0.088**
GLP-2 (ng/ml)			−0.139	0.87	0.100	0.71
Oxm (pg/ml)			**−0.400**	**0.072**	**−0.641**	**0.004**
Glicentin (pmol/l)			**−0.475**	**0.029**	**−0.767**	**<0.001**
Glucagon (pg/ml)			0.038	0.87	−0.065	0.79
MPGF (ng/ml)			−0.152	0.51	−0.294	0.24
PYY (pg/ml)			−0.292	0.18	−0.174	0.46
Ghrelin (pg/ml)			−0.029	0.93	−0.275	0.36
12						
GIP (pg/ml)					0.169	0.52
GLP-1 (pg/ml)					−0.054	0.83
GLP-2 (ng/ml)					0.197	0.45
Oxm (pg/ml)					−0.135	0.58
Glicentin (pmol/l)					−0.273	0.27
Glucagon (pg/ml)					0.214	0.37
MPGF (ng/ml)					0.030	0.91
PYY (pg/ml)					0.020	0.93
Ghrelin (pg/ml)					**−0.544**	**0.058**

Median with first and third quartile values are demonstrated. Post-hoc Dunn's multiple comparison tests were performed between all subject groups at the same month; "Sig." indicates $p < 0.05$ in post-hoc *t*-test between the % change of AUC of the hormone of the row where the number is written, with the % change of AUC of the hormone indicated by the number, e.g. for GIP at 3 months after operation, Sig. shows the number 4 and 5 meaning that the % change of AUC of GIP is significantly different from the % change of AUC of hormone 4, i.e. oxyntomodulin and of hormone 5, i.e. glicentin. *R* is the correlation coefficient and *Pa* the p value of this correlation. Parameters with $p < 0.10$ are marked bold.

AUCs of MPGF and GIP were observed only in the subjects that have undergone a RYGB (s. Fig. 2j,p and f,k), whereas the AUC of ghrelin was lower only in the VSG group (s. Fig. 2l,r).

Oxyntomodulin and glicentin demonstrated the most robust changes post-operatively (s. Table 1) (227–346% increase in AUC), followed by PYY (~53–69%), GLP-2 (~25–54%) and GLP-1 (~24–42%). GIP and MPGF demonstrated the most robust decreases (up to 32% and 29% respectively) similar to ghrelin (up to 19%), and these changes were dependent on the type of operation and anatomic changes thereof (RYGB for GIP and MPGF, and VSG for ghrelin).

3.3. Changes in Circulating Oxyntomodulin and Glicentin During MMT Three or Six Months After Operation Are Associated Prospectively With Weight Loss

Among the nine GI hormones, the AUC changes (in percent) in MMT at the third postoperative month significantly correlated with the weight changes (in percent) observed six months after surgery for GLP-1, oxyntomodulin, glicentin and MPGF or with the weight changes (in percent) twelve months after surgery for oxyntomodulin and glicentin (s. Table 1).

For the hormones (i.e. oxyntomodulin, glicentin, GLP-1 and MPGF) that demonstrated significant associations with weight loss, a linear regression analysis to investigate the predictive value of the hormonal changes in the first postoperative months on body weight loss six months or one-year after surgery was performed for each hormone was performed (s. Table 2). The linear regression analysis demonstrated that the AUC change (in percent) in MMT of glicentin and oxyntomodulin the first three or six months after operation are the most powerful predictor of weight changes (in percent) after six and/or twelve months postoperatively (s. Table 2), whereas weaker associations were observed for GLP-1.

A spearman correlation analysis was also performed for the AUC changes (in percent) of the four above hormones with changes (in percent) of parameters of adiposity, blood pressure and lipid profile (s. Table 2). None of the changes in the four hormones correlated with

Table 2
Linear regression of the relative changes of GLP-1, oxyntomodulin, glicentin and MPGF with weight changes and Spearman correlation coefficients of % changes of AUC after operation for these four hormones with % changes in parameters of adiposity, blood pressure and lipid profile in Study 2.

Linear Regression	→R^2 Intercept Unstand. B Stand. B CI for B p-value					
	All subjects					
Weight 6 months (% change after operation)						
AUC 3 months (% change)						
GLP-1 (pg/ml) (n = 22)	0.076	−25.233	−0.012	−0.275	−0.031, 0.007	0.216
Oxm (pg/ml) (n = 22)	0.285	−23.905	−0.003	−0.534	−0.005, −0.001	**0.010**
Glicentin (pmol/l) (n = 22)	0.164	−24.767	−0.002	−0.404	−0.003, 0.000	**0.062**
MPGF (ng/ml) (n = 21)	0.156	−25.255	0.010	0.395	−0.001, 0.022	**0.076**
Weight 12 months (% change after operation)						
AUC 3 months (% change)						
GLP-1 (pg/ml) (n = 20)	0.158	−31.939	−0.028	−0.398	−0.059, 0.004	**0.082**
Oxm (pg/ml) (n = 20)	0.192	−30.876	−0.004	−0.439	−0.008, 0.000	**0.053**
Glicentin (pmol/l) (n = 20)	0.198	−31.384	−0.003	−0.445	−0.006, 0.000	**0.049**
MPGF (ng/ml) (n = 19)	0.142	−32.465	0.010	0.377	−0.004, 0.037	0.11
AUC 6 months (% change)						
GLP-1 (pg/ml) (n = 18)	0.290	−29.671	−0.052	−0.538	−0.095, −0.009	**0.021**
Oxm (pg/ml) (n = 18)	0.420	−29.169	−0.006	−0.648	−0.009, −0.002	**0.004**
Glicentin (pmol/l) (n = 18)	0.488	−28.709	−0.007	−0.699	−0.011, −0.003	**0.001**
MPGF (ng/ml)	0.136	−31.270	0.019	0.369	−0.007, 0.046	0.15

Parameters (% change)	AUC (% Change after operation)					
Months	GLP-1 (pg/ml)			Oxm (pg/ml)		
	3	6	12	3	6	12
12						
FM (kg)	−0.148	−0.042	0.121	−0.060	0.028	0.093
FFM (kg)	0.077	0.126	−0.060	0.005	0.021	−0.082
SBP (mmHg)	−0.417	−0.491	−0.**600**†/−0.540	**−0.683**†/**−0.741***	**−0.600**†/−0.182	**−0.661***/−0.381
DBP (mmHg)	−0.600	**−0.758***/**0.610**†	−0.394	−0.583	**−0.709***/**−0**.151	−0.297
TG (mg/dl)	0.361	**0.480**†/**0**.354	0.059	0.305	0.407	0.035
TCH (mg/dl)	0.194	0.317	0.067	0.002	0.121	0.039
LDL (mg/dl)	0.144	0.282	0.119	−0.032	0.094	0.016
HDL (mg/dl)	−0.040	−0.310	−0.378	−0.176	**−0.524***/**0**.121	0.293

Parameters (% change)	AUC (% Change after operation)					
Months	MPGF (ng/ml)			Glicentin (pmol/l)		
	3	6	12	3	6	12
12						
FM (kg)	−0.196	0.027	0.280	−0.049	0.027	0.077
FFM (kg)	0.217	0.118	−0.098	0.005	−0.055	<0.001
SBP (mmHg)	**−0.738***/**−0.862****	**−0.733***/**−0.865***	**−0.850****/**−0.877****	**−0.700***/−0.589	−0.**576**†/−0.395	**−0.721***/−0.430
DBP (mmHg)	−0.548	**−0.767***/−0.613	−0.300	−0.483	**−0.685***/−0.390	−0.358
TG (mg/dl)	**0.476***/0.179	**0.632***/**0**.397	0.005	0.254	0.390	0.086
TCH (mg/dl)	0.212	0.397	0.228	−0.089	0.002	−0.089
LDL (mg/dl)	0.118	0.324	0.259	−0.077	−0.005	−0.112
HDL (mg/dl)	−0.065	−0.316	−0.222	−0.274	**−0.573****/**0**.123	−0.356

*, p value < 0.05; **, p value < 0.01; †, p-value = .05-0.088 AUC, area under the curve; Unstand. B, unstandardized Beta; Stand. B, Standardized Beta; CI, Confidence Interval. Among the nine hormones, GLP-1, oxyntomodulin, glicentin and MPGF were selected for linear regression, since they demonstrated significant associations with weight changes in the spearman correlation analysis. Significant values or trends are marked bold and after "/" is reported the correlation coefficient after adjusting for body weight changes at 12 months.

changes in parameters of adiposity. In contrast, there were several negative correlations of the relative changes of all the hormones with SBP or DBP. These associations lost significance after adjusting for weight loss, except from the negative association of the relative changes of MPGF with SBP which was maintained.

3.4. Feeling of Satiety Is Improved Postoperatively and Is Associated With the Circulating Levels of Oxyntomodulin and Glicentin

Feeling of satiety was assessed with a VAS scale (0−100) during a MMT. The AUC of VAS scale was increased during a MMT postoperatively, mainly in the VSG group (s. Supplemental Fig. 3, p.18 in Supplemental material). GLP-1 concentrations during a MMT both before as well as after operation correlate with VAS satiety score (s. Table 3). Interestingly, the concentrations of oxyntomodulin, glicentin and MPGF do not correlate with VAS satiety before operation, but they correlate strongly in the first six months after operation (Table 3). Preoperatively, only GLP-1 concentrations tend to correlate with insulin concentrations during MMT. After operation, an improved association mainly of GLP-1 and MPGF and secondarily of oxyntomodulin and glicentin with insulin concentrations during MMT is observed. Finally, hormones demonstrated strong collinearity with the strongest being observed between oxyntomodulin and glicentin. Interestingly, the strong positive associations of GLP-1, oxyntomodulin and glicentin with MPGF before operation become much weaker postoperatively.

4. Discussion

Our study reveals several novel findings. First, it shows a robust increase in postprandial levels of oxyntomodulin and glicentin after bariatric operation (RYGB or VSG but not AGB), which is more profound compared to the changes observed in GLP-1, GLP-2, PYY or any of the well-studied GI hormones. Second, the increase in oxyntomodulin and glicentin (% change of AUC) the first three months after operation is associated with weight loss (% change of weight) twelve months after surgery, much more than the established GLP-1, indicating possible utility of the above as biomarkers and/or possible direct involvement of these hormones in the processes leading to metabolic improvement. Third, in line with the above findings, feeling of satiety is increased and is associated with the concentrations of oxyntomodulin and glicentin. Altogether, our findings indicate an important role for oxyntomodulin and glicentin as biomarkers and possibly, through affecting satiety, in the regulation of energy homeostasis in humans.

Table 3
Spearman or Pearson correlation coefficients of GLP-1, oxyntomodulin, glicentin and MPGF with VAS satiety score and insulin before (0 months) and three, six and twelve months after operation in study 2.

Parameters	Months	Hormones (in all timepoints of MMT)			
(In all timepoints of MMT)		GLP-1 (pg/ml)	Oxm (pg/ml)	Glicentin (pmol/l)	MPGF (ng/ml)
VAS Satiety	0	**0.243*****	0.082	0.059	0.125
	3	**0.346****	**0.328*****	**0.309*****	**0.247****
	6	**0.250****	**0.410*****	**0.328*****	**0.185***
	12	**0.253****	0.156	0.150	**0.178***
Insulin (mlU/l)	0	**0.132**†	−0.087	0.017	0.083
	3	**0.332*****	0.106	**0.212****	**0.345*****
	6	**0.286*****	0.111	0.111	**0.288*****
	12	**0.436*****	**0.343*****	**0.370*****	**0.506**
GLP-1 (pg/ml)	0		**0.590*****	**0.535*****	**0.730*****
	3		**0.606*****	**0.648*****	**0.317*****
	6		**0.567*****	**0.538*****	**0.276****
	12		**0.810*****	**0.799*****	**0.552*****
Oxm (pg/ml)	0			**0.865*****	**0.656*****
	3			**0.953*****	**0.204***
	6			**0.888*****	**0.276*****
	12			**0.971*****	**0.271****
Glicentin (pmol/l)	0				**0.601*****
	3				**0.281*****
	6				**0.301*****
	12				**0.240****

* p-value < 0.05.
** p-value < 0.01.
*** p-value < 0.001.
† p-value = 0.07.

Previous studies have demonstrated that AGB has a minor to no effect on fasting or postprandial GLP-1, GLP-2, GIP, PYY and glucagon [10,11], while ghrelin levels are modestly increased post-operatively (reviewed in [12,13]). These findings were confirmed herein and were extended by showing for the first time no changes in fasting and postprandial circulating levels of oxyntomodulin, glicentin, and MPGF. Thus, except for the expected change in ghrelin, produced by the stomach, AGB does not alter the circulating levels of other metabolically important GI hormones.

In contrast to AGB, we observed robust changes in most of the GI hormones after RYGB or VSG. Previous studies have reported postprandial increase of GLP-1, GLP-2 and PYY both after VSG and RYGB [12–14]. We confirm these findings; somewhat lesser degree of improvements in postprandial GLP-1 profile could be attributed to differences in MMT composition, i.e. higher fat content (59%) vs. carbohydrates (33%) herein. Additionally, the changes in GLP-1 observed herein were more profound and significant in VSG than in RYGB group, in contrast to previous studies suggesting a more robust increase after RYGB than VSG [15,16]. Our study included mainly morbidly obese individuals (BMI ~49 kg/m^2) without T2D whereas other trials included obese patients with BMI 39–42 kg/m^2 and T2D. There were no differences in metabolic parameters before operation between our VSG and RYGB groups, limiting thus any possibility for selection bias due to these reasons.

No GIP changes were observed after VSG vs. a reduction after RYGB. These findings agree with previous reports suggesting that the restriction of nutrient passage through the duodenum and jejunum after RYGB may affect the postprandial secretion of GIP from K cells of the mucosa [10,17]. The lack of a robust increase of GIP in VSG argues against a significant contribution of this hormone in the metabolic benefit observed post-operatively. Ghrelin in our study was profoundly reduced after VSG and remained unchanged after RYGB. Ghrelin is secreted by the ghrelinergic cells located primarily in gastric fundus and the surgical removal of the fundus during VSG may explain the robust reduction in the circulating levels of the hormone [18]. Although it is clear that ghrelin decreases after VSG, contradictory results have been reported after RYGB, with some suggesting a reduction and others an increase of ghrelin after operation [19,20]. We did not observe any robust changes in ghrelin after RYGB, arguing against a major role of this hormone in postoperative metabolic changes.

Glucagon and MPGF are hormones secreted by the pancreas that derive from the post-translational processing of proglucagon peptide. Specifically, MPGF consists of the 72–158 aminoacids of the proglucagon-peptide and glucagon from 33 to 61 aminoacids. MPGF has been considered a by-product of the proglucagon peptide so far with unknown functional relevance and a precursor of GLP-1 (72-107/8 aminoacids) and GLP-2 (126–158). Circulating levels of MPGF after bariatric surgery have not been assessed so far, while very few studies have investigated the effect of bariatric surgery on glucagon concentrations, reporting contradictory results [10,21,22]. In our study, glucagon levels did not change after operation. In contrast, MPGF is detected in high concentrations in the circulation, its levels do not change after meal intake, but they are reduced after RYGB. This finding warrants further mechanistic studies to investigate possible role of MPGF, per se or as a source of active metabolites, in metabolic processes.

Oxyntomodulin and glicentin were the two gastrointestinal hormones that demonstrated the most robust changes after bariatric operation. Both hormones are secreted from the L cells of the gut and derive

from the proglucagon peptide, i.e. glicentin consists of 1–69 and oxyntomodulin of 33–69 aminoacids of the proglucagon peptide. Oxyntomodulin binds both to the GLP-1 and glucagon receptor, thus it is considered a dual agonist [23]. Consistent with dual binding properties it stimulates insulin secretion, and may reduce energy intake through binding to the GLP-1 receptor and it may increase energy expenditure through binding to glucagon-receptor in humans [24,25]. Thus it may both improve glucose levels and reduce weight [26]. Glicentin is much more novel and has been less investigated so far. Although in animals glicentin stimulates insulin secretion, reduces gut motility and gastric acid secretion and stimulates gut growth, a specific receptor for the hormone remains to be identified [27]. A previous study has reported increased fasting glicentin levels after RYGB and VSG (in that study no postprandial levels were reported) [28], whereas another study demonstrated higher glicentin levels 30 min after OGTT in lean individuals that have undergone total gastrectomy with Roux-en-Y reconstruction compared to lean healthy controls [29]. Our study is the first to show a robust postprandial increase of glicentin in morbidly obese individuals both after RYGB and VSG but not after AGB. This robust increase is achieved the first three months of the operation and is maintained for at least one year. Most importantly, the changes in glicentin not only precede but can, at least partially, predict the expected weight loss after one year (better than GLP-1). According to the linear regression models, up to almost 50% of the variation in weight loss observed after bariatric surgery may be attributed to early changes in the concentrations of glicentin.

To further elucidate potential underlying mechanisms we studied alterations in satiety in relation to improvements in postprandial responses to oxyntomodulin or glicentin. We report that concentration of glicentin and oxyntomodulin assessed 3 and 6 months post operatively correlate with satiety significantly and equally or even stronger than GLP-1. In contrast, GLP-1 levels are consistently much stronger associated with insulin levels compared to glicentin and oxyntomodulin, confirming the more profound established role of GLP-1 on beta cell function and insulin release.

The number of subjects was relatively small but given the strength of associations observed with GLP-1, which could be seen as a positive control, and oxyntomodulin and glicentin, the power of the study proved to be adequate. It would have been informative to have measured energy intake as well as metabolic rate using metabolic chambers but these more expensive and invasive methods are not readily available. Nevertheless, the current study provides a comprehensive analysis of novel and established gastrointestinal hormones in two independent clinical studies and with assays that have been well validated. Finally, measurements for this study were performed blindly by personnel that had no knowledge of the study hypotheses. All these increase confidence in the validity and reproducibility of study findings.

4.1. Clinical Implications

Changes in hormone levels observed herein could be used as biomarkers of success after bariatric surgery and could inform rational selection of hormonal agonists (based on incretins or other gastrointestinal hormones) to be used in combination for the treatment of diabetes and/or obesity [5,30]. Several on-going clinical trials are evaluating the efficacy of oxyntomodulin or other novel-peptides with GLP-1/glucagon receptor co-agonist properties in overweight or obese subjects with or without diabetes [31]. Based on our findings, we expect these studies to be more fruitful. There are no on-going clinical trials with glicentin, whereas important functional properties of glicentin (e.g. its receptor) remain largely unknown. Our findings indicate that glicentin may also be an attractive therapeutic target against obesity and further mechanistic studies to decipher its molecular targets and pathways underlying its role in metabolism are needed.

Declaration of Competing Interest

CSM is advisor of Ansh Labs LLC, is consultant to Intarcia and grant recipient through BIDMC and consultant to Novo Nordisk.

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Author contributions: C.S.M designed the experiment. All authors contributed to the performance of the experiments and acquisition of the data. Ni.P analyzed the data and wrote the manuscript, which was critically revised by all the other authors. The final version of the manuscript was approved by all authors. Ni.P is the guarantor of the current study.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.153997.

References

[1] Holst JJ, Madsbad S. Mechanisms of surgical control of type 2 diabetes: GLP-1 is key factor. Surg Obes Relat Dis 2016;12(6):1236–42.
[2] Phillips BT, Shikora SA. The history of metabolic and bariatric surgery: development of standards for patient safety and efficacy. Metabolism 2018;79:97–107.
[3] Smith EP, Polanco G, Yaqub A, Salehi M. Altered glucose metabolism after bariatric surgery: what's GLP-1 got to do with it? Metabolism 2018;83:159–66.
[4] Upadhyay J, Polyzos SA, Perakakis N, Thakkar B, Paschou SA, Katsiki N, et al. Pharmacotherapy of type 2 diabetes: an update. Metabolism 2018;78:13–42.
[5] Frias JP, Bastyr 3rd EJ, Vignati L, Tschop MH, Schmitt C, Owen K, et al. The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. Cell Metab 2017;26(2) (343-52 e2).
[6] Srivastava G, Apovian C. Future pharmacotherapy for obesity: new anti-obesity drugs on the horizon. Curr Obes Rep 2018;7(2):147–61.
[7] Drucker DJ, Habener JF, Holst JJ. Discovery, characterization, and clinical development of the glucagon-like peptides. J Clin Invest 2017;127(12):4217–27.
[8] Perakakis N, Kokkinos A, Peradze N, Tentolouris N, Ghaly W, Tsilingiris D, et al. Follistatins in glucose regulation in healthy and obese individuals. Diabetes Obes Metab 2019;21(3):683–90.
[9] Kokkinos A, Alexiadou K, Liaskos C, Argyrakopoulou G, Balla I, Tentolouris N, et al. Improvement in cardiovascular indices after Roux-en-Y gastric bypass or sleeve gastrectomy for morbid obesity. Obes Surg 2013;23(1):31–8.
[10] Korner J, Bessler M, Inabnet W, Taveras C, Holst JJ. Exaggerated glucagon-like peptide-1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis 2007;3(6):597–601.
[11] Shak JR, Roper J, Perez-Perez GI, Tseng CH, Francois F, Gamagaris Z, et al. The effect of laparoscopic gastric banding surgery on plasma levels of appetite-control, insulinotropic, and digestive hormones. Obes Surg 2008;18(9):1089–96.
[12] Dimitriadis GK, Randeva MS, Miras AD. Potential hormone mechanisms of bariatric surgery. Curr Obes Rep 2017;6(3):253–65.
[13] Meek CL, Lewis HB, Reimann F, Gribble FM, Park AJ. The effect of bariatric surgery on gastrointestinal and pancreatic peptide hormones. Peptides 2016;77:28–37.
[14] Laferrere B, Swerdlow N, Bawa B, Arias S, Bose M, Olivan B, et al. Rise of oxyntomodulin in response to oral glucose after gastric bypass surgery in patients with type 2 diabetes. J Clin Endocrinol Metab 2010;95(8):4072–6.
[15] Casajoana A, Pujol J, Garcia A, Elvira J, Virgili N, de Oca FJ, et al. Predictive value of gut peptides in T2D remission: randomized controlled trial comparing metabolic gastric bypass, sleeve gastrectomy and greater curvature plication. Obes Surg 2017;27(9):2235–45.
[16] Nosso G, Griffo E, Cotugno M, Saldalamacchia G, Lupoli R, Pacini G, et al. Comparative effects of Roux-en-Y gastric bypass and sleeve gastrectomy on glucose homeostasis and incretin hormones in obese type 2 diabetic patients: a one-year prospective study. Horm Metab Res 2016;48(5):312–7.
[17] Bunt JC, Blackstone R, Thearle MS, Vinales KL, Votruba S, Krakoff J. Changes in glycemia, insulin and gut hormone responses to a slowly ingested solid low-carbohydrate mixed meal after laparoscopic gastric bypass or band surgery. Int J Obes (Lond) 2017;41(5):706–13.
[18] Yousseif A, Emmanuel J, Karra E, Millet Q, Elkalaawy M, Jenkinson AD, et al. Differential effects of laparoscopic sleeve gastrectomy and laparoscopic gastric bypass on appetite, circulating acyl-ghrelin, peptide YY3-36 and active GLP-1 levels in non-diabetic humans. Obes Surg 2014;24(2):241–52.

[19] Dirksen C, Jorgensen NB, Bojsen-Moller KN, Kielgast U, Jacobsen SH, Clausen TR, et al. Gut hormones, early dumping and resting energy expenditure in patients with good and poor weight loss response after Roux-en-Y gastric bypass. Int J Obes (Lond) 2013;37(11):1452–9.
[20] Yang J, Feng X, Zhong S, Wang Y, Liu J. Gastric bypass surgery may improve beta cell apoptosis with ghrelin overexpression in patients with BMI >/= 32.5 kg/m(2.). Obes Surg 2014;24(4):561–71.
[21] Farey JE, Preda TC, Fisher OM, Levert-Mignon AJ, Stewart RL, Karsten E, et al. Effect of laparoscopic sleeve gastrectomy on fasting gastrointestinal, pancreatic, and adipose-derived hormones and on non-esterified fatty acids. Obes Surg 2017;27(2):399–407.
[22] Umeda LM, Silva EA, Carneiro G, Arasaki CH, Geloneze B, Zanella MT. Early improvement in glycemic control after bariatric surgery and its relationships with insulin, GLP-1, and glucagon secretion in type 2 diabetic patients. Obes Surg 2011;21(7):896–901.
[23] Pocai A, Carrington PE, Adams JR, Wright M, Eiermann G, Zhu L, et al. Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 2009;58(10):2258–66.
[24] Hope DCD, Tan TMM, Bloom SR. No guts, no loss: toward the ideal treatment for obesity in the twenty-first century. Front Endocrinol (Lausanne) 2018;9:442.
[25] Wynne K, Park AJ, Small CJ, Meeran K, Ghatei MA, Frost GS, et al. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. Int J Obes (Lond) 2006;30(12):1729–36.
[26] Shankar SS, Shankar RR, Mixson LA, Miller DL, Pramanik B, O'Dowd AK, et al. Native oxyntomodulin has significant glucoregulatory effects independent of weight loss in obese humans with and without type 2 diabetes. Diabetes 2018;67(6):1105–12.
[27] Raffort J, Lareyre F, Massalou D, Fenichel P, Panaia-Ferrari P, Chinetti G. Insights on glicentin, a promising peptide of the proglucagon family. Biochem Med (Zagreb) 2017;27(2):308–24.
[28] Raffort J, Panaia-Ferrari P, Lareyre F, Bayer P, Staccini P, Fenichel P, et al. Fasting circulating glicentin increases after bariatric surgery. Obes Surg 2017;27(6):1581–8.
[29] Roberts GP, Kay RG, Howard J, Hardwick RH, Reimann F, Gribble FM. Gastrectomy with Roux-en-Y reconstruction as a lean model of bariatric surgery. Surg Obes Relat Dis 2018;14(5):562–8.
[30] Frias JP, Nauck MA, Van J, Kutner ME, Cui X, Benson C, et al. Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. Lancet 2018;392:2180–93.
[31] Tillner J, Posch MG, Wagner F, Teichert L, Hijazi Y, Einig C, et al. A novel dual glucagon-like peptide and glucagon receptor agonist SAR425899: results of randomized, placebo-controlled first-in-human and first-in-patient trials. Diabetes Obes Metab 2018;21:120–8.