RESEARCH

Response of multiple hormones to glucose and arginine challenge in T2DM after gastric bypass

Giovanni Fanni[1], **Petros Katsogiannos**[1], **Bipasha Nandi Jui**[1], **Magnus Sundbom**[2], **Susanne Hetty**[1], **Maria J Pereira**[1] and **Jan W Eriksson**[1]

[1]Department of Medical Sciences, Clinical Diabetes and Metabolism, Uppsala University, Uppsala, Sweden
[2]Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

Correspondence should be addressed to J Eriksson: jan.eriksson@medsci.uu.se

Abstract

Purpose: In patients with type 2 diabetes mellitus (T2DM), Roux-en-Y gastric bypass (RYGB) leads to beneficial metabolic adaptations, including enhanced incretin secretion, beta-cell function, and systemic insulin sensitivity. We explored the impact of RYGB on pituitary, pancreatic, gut hormones, and cortisol responses to parenteral and enteral nutrient stimulation in patients with obesity and T2DM with repeated sampling up to 2 years after intervention.

Methods: We performed exploratory *post hoc* analyses in a previously reported randomized trial. Levels of adrenocorticotropic hormone (ACTH), cortisol, growth hormone (GH), glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP), peptide YY (PYY), ACTH, insulin, and glucagon were measured in 13 patients with T2DM and obesity at four different visits: before and 4, 24, and 104 weeks after RYGB; and in three sequential conditions on the same day: fasting, intravenous arginine challenge, and OGTT.

Results: RYGB surprisingly induced a rise in ACTH, cortisol, and GH levels upon an oral glucose load, together with enhanced GLP-1 and PYY responses. Fasting and post-arginine GH levels were higher after RYGB, whereas insulin, glucagon, GLP-1, GIP, and cortisol were lower. These endocrine adaptations were seen as early as 4 weeks after surgery and were maintained for up to 2 years.

Conclusion: These findings indicate adaptations of glucose sensing mechanisms and responses in multiple endocrine organs after RYGB, involving the gut, pancreatic islets, the pituitary gland, the adrenals, and the brain.

Key Words
- diabetes
- RYGB
- nutrient challenge
- gut hormones
- HPA-axis

Endocrine Connections (2022) **11**, **e220172**

Introduction

Besides inducing significant and durable weight loss, Roux-en-Y gastric bypass (RYGB) improves glycemic control in insulin-resistant patients and can prevent or reverse type 2 diabetes mellitus (T2DM) (1, 2, 3). This metabolic shift is partly independent of weight loss, and the underlying mechanisms are not completely understood (3, 4, 5, 6). Shortly after RYGB, a greater incretin response occurs post-prandially, which enhances insulin secretion, reduces food intake, and contributes to improved systemic insulin sensitivity (7, 8). This is related to the rearranged gastrointestinal anatomy resulting in rapid transport of ingested nutrients to the small intestine (3). However, CNS and neuroendocrine pathways have been suggested to play a role in mediating the effects of RYGB on glucose homeostasis (9, 10).

Intravenous administration of an L-arginine bolus is a well-established technique for assessing beta-cell secretion capacity (11). It also has potent secretagogue effects on

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pancreatic alpha-cells, gut L-cells, and anterior pituitary somatotrope cells (12, 13, 14, 15, 16), but not on ACTH-producing anterior pituitary cells (17). Notably, little is known about whether RYGB affects endocrine responses to this aminoacidic stimulus (18).

The oral glucose tolerance test (OGTT) is a standardized technique to assess the metabolic and overall hormonal response to an oral glucose load and is a validated diagnostic tool for impaired glucose tolerance, T2DM, and gestational diabetes mellitus (19).

We have recently reported rapid effects on neuroendocrine regulation following RYGB (8) as well as early and late adipose tissue effects in patients with obesity and T2DM (9, 20). We have now performed additional exploratory analyses in the same cohort, and the current work aimed to explore the changes in the dynamic endocrine response induced by RYGB in patients with T2DM using two different nutrient challenges, namely intravenous arginine challenge and OGTT. RYGB-induced neuroendocrine adaptations might be different during nutrient challenges as compared to fasting, and we hypothesize that RYGB may not only alter nutrient-induced secretion of hormones secreted by the gut and pancreatic islets but also of others produced by the pituitary and adrenal glands. Therefore, in this study, we assess growth hormone (GH), ACTH, and cortisol levels, which are largely unexplored in this context. Herein, we report exploratory *post hoc* analyses of multiple hormones in patients with obesity and T2DM followed up for 2 years after RYGB. We highlight the novel findings on the responses of GH and the hypothalamus–pituitary–adrenal (HPA) axis to oral glucose following RYGB. For the first time in this context, we also characterize responses to intravenous arginine stimulation.

Materials and methods

Study design and ethics

This exploratory *post hoc* analysis is part of a previously described randomized controlled trial (8, 9, 20) carried out in 19 patients (18–65 years, BMI: 30–45 kg/m^2) with T2DM, diagnosed less than 10 years before the study entry and treated with oral antidiabetic medication (Supplementary Table 2, see section on supplementary materials given at the end of this article). The subjects were randomly assigned 2:1 to RYGB or standard-of-care medical treatment without any other weight-lowering treatment. Further characteristics of this cohort are presented in Supplementary Table 1 and have been reported previously (8, 9, 20). Data on other neuroendocrine responses from this cohort in a shorter follow-up have been previously reported, together with fasting data (8, 9).

The study (clinicaltrials.gov NCT02729246) was conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethics Review Board in Uppsala (Dnr 2014/255). All participants had given their written informed consent before enrolment.

Study procedures

The 13 patients who underwent RYGB were studied at four-time points: (i) before surgical intervention (pre-surgery visit); (ii) 4 weeks; (iii) 24 weeks; (iv) 104 weeks after intervention. Data from the six patients belonging to the control group were obtained only at the first visit (baseline) and at 24 weeks. Anthropometric measurements (weight, waist/hip circumference, and bioimpedance for body fat measurement), subcutaneous adipose tissue biopsies, OGTT, and arginine challenge were performed in 1-day visits after an overnight fast. Individuals randomized to surgery followed a low-calorie diet (LCD, 3350–4600 kJ/day) for 4 weeks after the pre-surgery visit before undergoing surgical intervention, according to clinical routine. After RYGB, antidiabetic medications were reduced or withdrawn, and other medication changes were made as clinically appropriate (9).

Blood samples were collected for hormonal measurements in the morning starting at 08:00 h under the following conditions and in this sequence: (i) after overnight fast; (ii) 3 min after administrating an intravenous bolus dose of arginine 5 g (infused over 15–20 s); (iii) during a 3 h OGTT (75 g) with sampling at 0, 15, 30, 60, 90, 120, and 180 min, starting 30 min after arginine administration. The arginine challenge was first used to assess functional beta-cell reserve. According to the original protocol, samples were obtained 3 min after 5 g arginine bolus administration to assess beta-cell secretion capacity (11). The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), Matsuda insulin sensitivity index, and the insulinogenic indices were calculated.

Biochemical measurements

Assays and materials have been described previously (20). Plasma and serum samples for all assessments, except those immediately performed, were frozen and stored at –80°C. For analyses, commercially available ELISA or multiplex kits were used: glucagon and glicentin (Mercodia, Uppsala,

Sweden); total glucagon-like peptide (GLP)-1 (7-36 and 9-36) and total glucose-dependent insulinotropic peptide (GIP) (Merck Millipore, Darmstadt, Germany); peptide YY (PYY) and GH (Meso Scale Discovery, Rockville, USA); or with routine methods at the Uppsala University Hospital chemical laboratory (ACTH, cortisol). All protocols were followed according to the manufacturers' instructions. The glucagon measurements were performed both with the previously used simultaneous assay protocol ($n = 13$) (21) and with the improved-specificity sequential protocol, as reported by Roberts *et al.* ($n = 7$) (22). The glicentin assay was performed on OGTT samples only taken before surgery and at 4 and 104 weeks after RYGB ($n = 5$).

Statistical analyses

Analyses of variance across visits were performed with mixed-effect models for glucose and all hormones. Areas under the curve for the OGTT (AUC_{OGTT}) were calculated using the trapezoidal rule. Values of missing samples at the first and last time point of the OGTT were interpolated from the mean of the adjacent measured values. Pairwise comparisons between follow-up and pre-surgery data for the RYGB and the control groups were performed with paired *t*-tests. Hormonal level comparisons between control and surgical patients were performed with independent *t*-tests. Correlation analyses were performed using Spearman's test. Data are presented as mean ± S.E. unless otherwise indicated. All analyses and calculations were performed using GraphPad Prism 9 and Microsoft Excel for Mac 16.

Results

Fasting and arginine challenge

Following RYGB, circulating levels of glucose, insulin, total glucagon-like peptide 1 (GLP-1), and GIP were reduced both in the fasting state and during the arginine challenge for the whole follow-up, while cortisol levels were reduced only at 4 weeks after surgery. Glucagon levels were significantly decreased only during fasting (Fig. 1 and Supplementary Table 3). Arginine infusion significantly enhanced secretion of insulin, glucagon, and total GLP-1 during the pre-surgical visit and throughout the follow-up compared to fasting. However, the arginine challenge-fold effect on all hormonal levels was unaffected by RYGB (Supplementary Fig. 2).

RYGB induced a significant rise in GH levels both in the fasting state and during the arginine challenge, with a maximal increase 24 weeks after surgery (Fig. 1 and Supplementary Table 3).

The variation of the hormonal levels during the arginine challenge 104 weeks after surgery was not associated with pre-surgical age, weight, BMI, waist-to-hip ratio, total body fat, HbA1c, HOMA-IR, Matsuda index, disposition index, or their 2-year post-surgical change (data not shown).

Thirty minutes after arginine administration (at the beginning of the OGTT), all hormonal values had returned to the fasting level; therefore, a carryover effect of previous arginine administration during the OGTT is unlikely.

The control subjects did not show changes in the level of any analyzed hormone (glucagon, GLP-1, GIP, GH, and PYY) during either fasting or arginine challenge between baseline and 24-week follow-up, with the exception of ACTH ($P = 0.03$ for both conditions). Also, the baseline hormonal values of the control patients were similar to the pre-surgery levels of the patients who underwent RYGB (data not shown).

OGTT

Glucose levels during the OGTT were characterized by an earlier peak, a more rapid decrease, and reduced AUC at post-surgery visits compared to pre-surgery (Supplementary Fig. 1). Dumping symptoms during the OGTT were experienced by seven, five, and two patients at the 4-week, 24-week, and 2-year follow-up visits, respectively.

We show significantly elevated total and incremental AUC_{OGTT} for ACTH and cortisol throughout the follow-up (Fig. 2 and Supplementary Fig. 3). This affected all patients independently of dumping symptoms (Supplementary Table 4). The enhanced HPA-axis responses remained stable throughout the 2-year follow-up period, whereas dumping episodes were markedly fewer over time. We also observed a significant increase in the AUC_{OGTT} for total GLP-1 and PYY in all follow-up visits after surgery compared to pre-surgery. We observed no correlation between GLP-1 and ACTH or cortisol levels in the post-surgery follow-up visits 30 min after glucose ingestion ($r = 0.108$, $P = 0.55$; $r = 0.131$, $P = 0.46$, respectively). Despite the curve shape alteration, the GIP AUC_{OGTT} was unchanged, which can be explained by an earlier and higher peak followed by a quicker fall of its plasma concentration. Furthermore, the total AUC_{OGTT} of GH was elevated throughout the follow-up, with the highest

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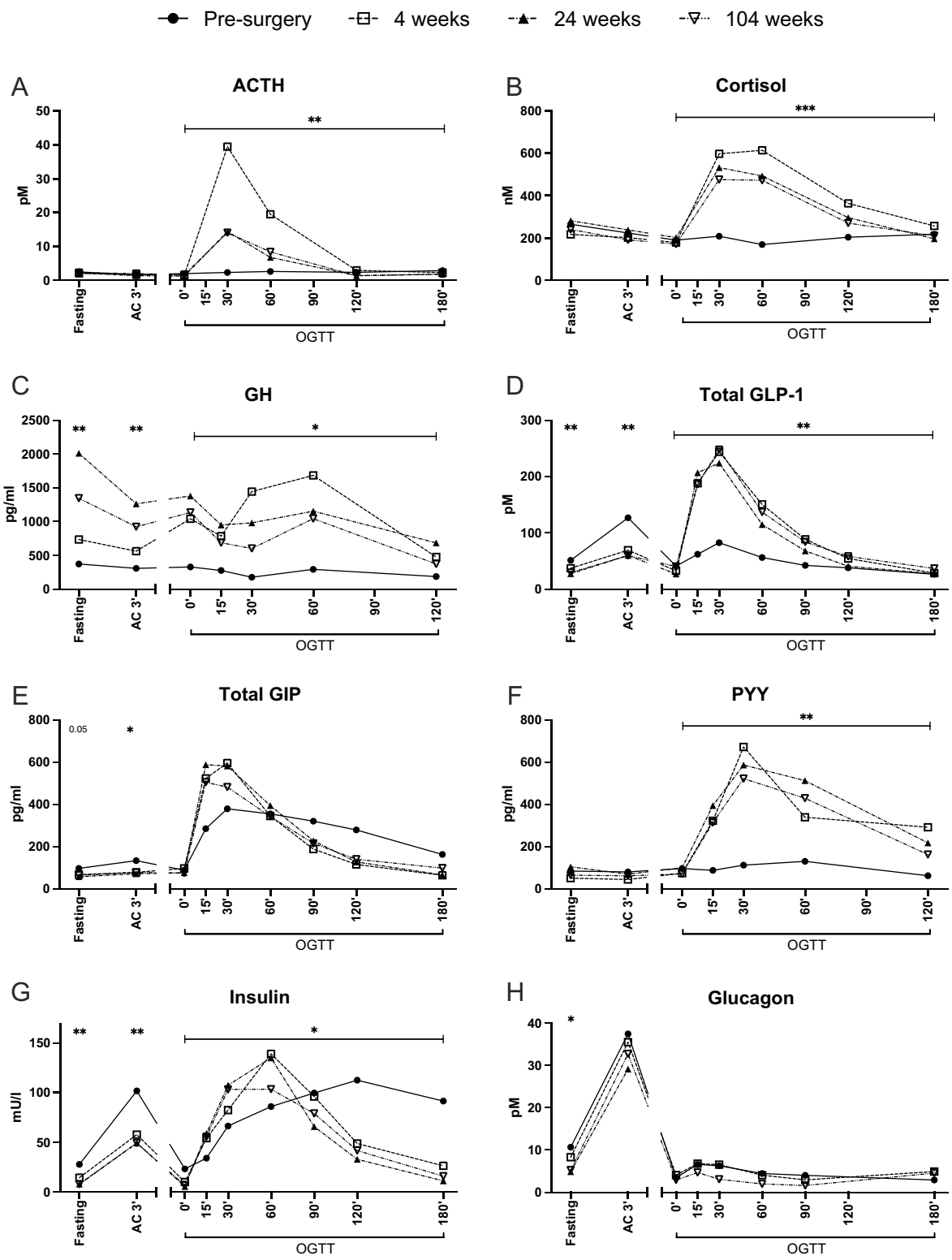

Figure 1

Plasma hormonal levels during fasting, 3 min after intravenous arginine administration (AC 3'), and OGTT in patients with obesity and type 2 diabetes before and 4, 24, and 104 weeks after RYGB. (A) ACTH, (B) cortisol, (C) GH, (D) total GLP-1, (E) total GIP, (F) PYY, (G) insulin, and (H) glucagon. Data are presented as mean. Mixed-effects models for differences in hormone levels or AUC_{OGTT} across visits: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. $N = 13$ (except for glucagon, $N = 7$). ACTH, adrenocorticotropic hormone; GH, growth hormone; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic polypeptide; PYY, peptide YY; RYGB, Roux-en-Y gastric bypass.

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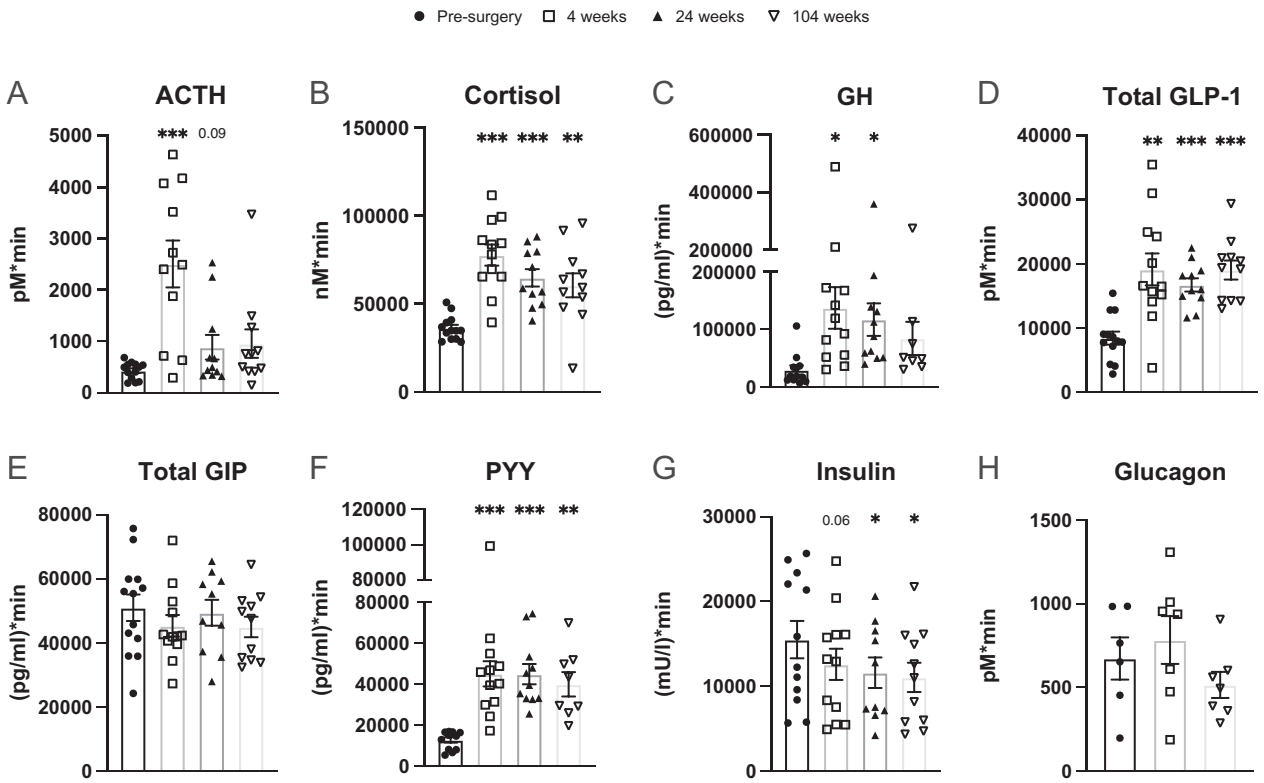

Figure 2

AUC_{OGTT} of plasma hormone levels in patients with obesity and type 2 diabetes before and 4, 24, and 104 weeks after RYGB. (A) ACTH, (B) cortisol, (C) GH, (D) total GLP-1, (E) total GIP, (F) PYY, (G) insulin, and (H) glucagon. Data presented as mean ± S.E.M. Pairwise comparisons for post-surgery AUC_{OGTT} with pre-surgery AUC_{OGTT} with paired *t*-tests. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. $N = 13$ (except for glucagon, $N = 7$). ACTH, adrenocorticotropic hormone; GH, growth hormone; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic polypeptide; PYY, peptide YY; RYGB, Roux-en-Y gastric bypass.

value at 4 weeks after surgery (Fig. 2). However, the GH incremental AUC_{OGTT} was unchanged, albeit with a decreasing trend (Supplementary Fig. 3).

The glucagon levels measured using the improved-specificity sequential protocol (see the 'Materials and methods' section) were slightly decreased during OGTT 2 years after RYGB vs pre-surgery (Fig. 2). The new glucagon assay did not replicate the previous findings that showed high glucagon levels after surgery when using the simultaneous assay protocol (8, 23). The difference can be explained by the previous assay's cross-reactivity with glicentin (24), which was markedly elevated after RYGB (Supplementary Fig. 4).

The change in any hormonal AUC_{OGTT} 2 years after surgery was not associated with pre-surgery age, weight, BMI, waist-to-hip ratio, total body fat, HbA1c, HOMA-IR, Matsuda index, disposition index, or their 2-year post-surgery change (data not shown).

The control patients did not show differences in AUC_{OGTT} of total GLP-1, GIP, GH, and PYY between the baseline and 24-week follow-up visits (data not shown).

Discussion

We assessed for the first time dynamic and parallel responses of pituitary, pancreatic, gut hormones, and cortisol during both an arginine challenge test and an OGTT in a cohort of patients with T2DM who underwent RYGB and in repeated follow-up visits up to 2 years after intervention (Table 1). Our results support that many of the endocrine changes seen in the first post-operative month are robustly sustained up to 2 years after the intervention, both during fasting and under nutrient stimulation.

HPA-axis and GH

Fasting cortisol levels were slightly reduced after RYGB, suggesting a lower adrenal counterregulatory drive in the fasting state after RYGB. We did not observe any secretagogue effect of intravenous arginine itself on the HPA-axis, as recently reported in healthy individuals (17).

GH secretion is impaired in basal conditions and upon stimulation in individuals with obesity (25, 26).

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Table 1 A comprehensive scheme of hormonal changes induced by RYGB in patients with obesity and T2DM 2 years after intervention.

Hormones	Fasting	3 min after intravenous arginine administration	AUC_{OGTT}
ACTH	⇔	⇔	↑
Cortisol	⇔	⇔	↑
GH	↑	↑	↑
Total GLP-1	↓	↓	↑
Total GIP	⇩	↓	⇔
PYY	⇔	⇔	↑
Insulin	⇩	⇩	↓
Glucagon	↓	⇔	⇔

Mixed-effects models for differences across visits. Down arrow: significant (black, $P < 0.05$) or nearly significant (white, $P < 0.10$) reduction. Horizontal arrow: no significant change ($P > 0.10$). Up arrow: significant (black, $P < 0.05$) or nearly significant (white, $P < 0.10$) increase.
ACTH, adrenocorticotropic hormone; GH, growth hormone; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic polypeptide; PYY, peptide YY.

We reported higher fasting levels of GH after surgery, which is consistent with the higher IGF-1 levels that we have previously shown (9). This is in line with previous findings in individuals without diabetes (27). The elevation in GH could be mediated by higher fasting levels of ghrelin, which is known to promote GH secretion (28). However, reduced ghrelin levels have been reported up to 3 months after RYGB (29), while we showed higher GH levels even 4 weeks after RYGB. Our data suggest that RYGB can restore GH-axis functionality via weight reduction and associated changes in body composition in patients with T2DM. Even though arginine is known to stimulate pituitary somatotrope cells (30), we did not see increased levels of GH during the arginine challenge. However, 3 min after the bolus might not be sufficient to detect its effect (31, 32). Standard protocols for eliciting GH response imply larger doses of arginine administrated continuously together with GH-releasing hormone for 30 min (31). In addition, arginine administration might have affected GH levels during OGTT. Indeed, the study procedure was repeated in the same manner throughout the follow-up, thus excluding biases in the assessment of RYGB on hormonal levels. Also, GH, ACTH, and cortisol are secreted in a pulsatile fashion, which might interfere with detecting consistent differences in their levels with our study design. However, since pulse periods are around 2 h (ACTH and cortisol) (32) and 3 h (GH) (33), it is unlikely that this variability interfered with the effects measured 3 min after arginine administration.

To the best of our knowledge, this is the first study assessing the effect of RYGB on the HPA-axis response to a glucose load. Total and incremental AUC_{OGTT} for both ACTH and cortisol were significantly increased, although a glucose load is known to suppress cortisol secretion, irrespectively of obesity or insulin resistance (34, 35, 36). This suggests that following RYGB, the HPA-axis became susceptible to stimulation by OGTT. A recent review has suggested a direct stimulatory effect of GLP-1 on the HPA-axis via enhanced secretion of corticotropin-releasing hormone (37). Of note, there are no published data on whether other hormonal responses are differentially affected by oral or intravenous glucose load after RYGB. However, preliminary results from an ongoing study of ours (38) indicate that in participants with obesity there were no changes in either ACTH and cortisol levels during a hyperglycemic clamp, and this was also unchanged after RYGB. This would argue that the rise in ACTH and cortisol in the present work following RYGB is specific to oral glucose administration, suggesting the relevance of the glucose–gut interaction in regulating the HPA-axis. However, we did not find any correlation between GLP-1 levels and ACTH and cortisol levels 30 min after glucose load (peak time) in the post-RYGB visits. Further studies are warranted to investigate whether incretins themselves are responsible for these post-RYGB endocrine adaptations. We cannot exclude that the HPA-axis activation was related to early dumping syndrome and its hemodynamic implications that activate the stress response, since there was a trend for higher ACTH and cortisol levels in patients who experienced early dumping syndrome symptoms during the glucose challenge.

GH AUC_{OGTT} was increased after RYGB, while GH incremental AUC_{OGTT} showed a decreasing trend, suggesting that the GH response to glucose was mainly dependent on higher fasting GH levels. Our result supports that weight reduction following RYGB can restore GH-axis responsiveness to various stimuli (38), even though much of the response was dependent on higher pre-OGTT fasting GH levels. It is possible that changes in ghrelin levels have influenced the GH response since ghrelin levels during the OGTT are known to be suppressed in post-RYGB individuals (39).

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Incretins

In the fasting condition, we observed reduced levels of total GLP-1 and total GIP after surgery, which were maintained up to 2 years after the intervention. Accordingly, previous findings identified both lower (40, 41, 42) or unchanged (43) fasting total GLP-1 levels after RYGB, either in individuals with or without diabetes.

Besides its known secretagogue effect on beta- and alpha-cells and on somatotrope anterior pituitary cells (15, 30), intravenous arginine has been shown to stimulate GLP-1 secretion both in euglycemic and dysglycemic individuals (44), independently of beta-cell function (12). During the arginine challenge, we found increased secretion of total GLP-1 and, to a lesser extent, of total GIP after RYGB, but the fold effect was not altered. On the contrary, arginine administration did not stimulate PYY secretion, either before or after surgery. Although GLP-1 and PYY are both secreted by L-cells, proximal gut cells predominantly secrete GLP-1 and no PYY (45). This, together with differential response to different secretory stimuli (46), could explain the different behavior of GLP-1 and PYY observed during arginine stimulation.

RYGB enhances oral glucose-induced secretion of GLP-1 and PYY, but not of GIP (47). Also, after RYGB, glucose becomes the dominant nutrient in stimulating several gut hormones compared to lipids and proteins (48). Our results are in line with this evidence and show that this occurs as early as 4 weeks after the intervention and is sustained for up to 2 years. The role of gut hormones in mediating insulin response to hyperglycemia was confirmed by studies that showed increased insulin secretion during an OGTT but not during an intravenous glucose tolerance test or a hyperglycemic clamp (49, 50). Moreover, PYY was identified as crucial in rescuing islet function after RYGB (51).

Even though the GIP AUC_{OGTT} did not change after RYGB, GIP levels rose and fell more rapidly after the glucose load. This may partly be explained by altered gut anatomy and food transit, boosting incretin secretion and subsequently insulin release from beta-cells in the early post-prandial phase (52), thus improving post-prandial glycemic control.

Islet hormones

Fasting levels of insulin and glucagon were reduced after RYGB. The stimulation of insulin and glucagon secretion by arginine administration assessed by the fold change from the fasting levels was slightly higher after surgery, but this was mainly driven by a reduction in the fasting levels of these hormones.

Glucagon levels were slightly suppressed during the OGTT 2 years after RYGB, probably because of reduced alpha-cell insulin resistance (53) and increased circulating levels of GLP-1, which is known to inhibit glucagon secretion (54). However, we cannot exclude that altered circulating amino acid levels after surgery might have affected glucagon levels (55). A paradoxical increase of glucagon secretion during OGTT after metabolic surgery was previously reported in animal (56) and human (49, 57) studies, also by our group (8). However, these results were obtained using a non-specific glucagon assay that cross-reacted with glicentin, a receptor-orphan proglucagon byproduct released by L-cells and with an unclear biological function (58). We also found extremely elevated glicentin levels during the OGTT in five subjects after RYGB. These findings underscore the importance of using an optimized specific glucagon assay protocol in gastric bypass patients and the need to reconsider previous evidence of post-RYGB glucagon levels produced with non-specific assay protocols.

Limitations

This study has some limitations. The *post hoc* design of these sub-analyses compelled us to use data and samples that were already available, which might have been taken for purposes different from the ones presented here. Also, the sample size was small, thus some analyses might be underpowered, and some results need to be validated in larger cohorts. The control group did not undergo any weight loss intervention, and the patients in the RYGB group underwent a LCD 4 weeks before the intervention. This makes it difficult to distinguish the strictly RYGB-related outcomes from the LCD at 4 weeks, as the combined action of surgery and diet may explain the effects found. Also, this study included only patients with T2DM, and future studies should address patient groups without T2DM. Finally, the study design did not allow direct comparisons of the hormonal responses to arginine challenge and OGTT with one another.

Conclusion

Our results suggest that RYGB leads to profound changes in multiple hormonal responses to OGTT, with more rapid or enhanced secretion of GLP-1, PYY, GIP, insulin, and surprisingly, also GH, ACTH, and cortisol in patients with

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obesity and T2DM. This corresponds with altered glucose sensing in several organs, including the gut, pancreatic beta-cells, the brain, and the pituitary gland. These endocrine adaptations occur as early as 4 weeks after surgery and are maintained up to 2 years after the intervention. This might contribute to the observed adaptations of nutrient responses and potentially also to the favorable metabolic effects of RYGB. However, the underlying mechanisms and the possible role for the antidiabetic effects of RYGB are not well understood, and further investigations are warranted.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-22-0172.

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.

Funding

This work was supported by research grants from the Swedish Diabetes Foundation (DIA2019-490), the Exodiab – Excellence of Diabetes Research in Sweden, the Ernfors Foundation, the Swedish Society for Medical Research, the P.O. Zetterling Foundation, the Novo Nordisk Foundation (NNF20OC0063864), the European Commission via the Marie Sklodowska Curie Innovative Training Network TREATMENT (H2020-MSCA-ITN-721236), and the Uppsala University Hospital ALF grants (Swedish Government research support).

Data availability

Data and study protocol can be made available by the authors upon request.

Author contribution statement

G F, P K, and J W E designed the study. G F, P K, M J P, and B N J collected and analyzed the data. G F, M J P, and J W E interpreted the data. G F wrote the manuscript, and M J P, S H, M S, and J W E critically revised the manuscript.

Acknowledgements

The authors want to thank all the participants in the study and the whole staff at Clinical Diabetology and Metabolism, Department of Medical Sciences, Uppsala University, and Uppsala University Hospital for making this research possible.

References

1 Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Aminian A, Brethauer SA, Navaneethan SD, Singh RP, Pothier CE, Nissen SE, *et al*. Bariatric surgery versus intensive medical therapy for diabetes – 5-year outcomes. *New England Journal of Medicine* 2017 **376** 641–651. (https://doi.org/10.1056/NEJMoa1600869)

2 Purnell JQ, Dewey EN, Laferrère B, Selzer F, Flum DR, Mitchell JE, Pomp A, Pories WJ, Inge T, Courcoulas A, *et al*. Diabetes remission status during seven-year follow-up of the longitudinal assessment of bariatric surgery study. *Journal of Clinical Endocrinology and Metabolism* 2021 **106** 774–788. (https://doi.org/10.1210/clinem/dgaa849)

3 Cornejo-Pareja I, Clemente-Postigo M & Tinahones FJ. Metabolic and endocrine consequences of bariatric surgery. *Frontiers in Endocrinology* 2019 **10** 626. (https://doi.org/10.3389/fendo.2019.00626)

4 Mingrone G & Cummings DE. Changes of insulin sensitivity and secretion after bariatric/metabolic surgery. *Surgery for Obesity and Related Diseases* 2016 **12** 1199–1205. (https://doi.org/10.1016/j.soard.2016.05.013)

5 Herzog K, Berggren J, Majdoub M al, Arroyo CB, Lindqvist A, Hedenbro J, Groop L, Wierup N & Spégel P. Metabolic effects of gastric bypass surgery: is it all about calories? *Diabetes* 2020 **69** 2027–2035. (https://doi.org/10.2337/db20-0131)

6 Yoshino M, Kayser BD, Yoshino J, Stein RI, Reeds D, Eagon JC, Eckhouse SR, Watrous JD, Jain M, Knight R, *et al*. Effects of diet versus gastric bypass on metabolic function in diabetes. *New England Journal of Medicine* 2020 **383** 721–732. (https://doi.org/10.1056/NEJMoa2003697)

7 Svane MS, Bojsen-Møller KN, Madsbad S & Holst JJ. Updates in weight loss surgery and gastrointestinal peptides. *Current Opinion in Endocrinology, Diabetes, and Obesity* 2015 **22** 21–28. (https://doi.org/10.1097/MED.0000000000000131)

8 Katsogiannos P, Kamble PG, Wiklund U, Sundbom M, Espes D, Hammar U, Karlsson FA, Pereira MJ & Eriksson JW. Rapid changes in neuroendocrine regulation may contribute to reversal of type 2 diabetes after gastric bypass surgery. *Endocrine* 2020 **67** 344–353. (https://doi.org/10.1007/s12020-020-02203-w)

9 Almby KE, Katsogiannos P, Pereira MJ, Karlsson FA, Sundbom M, Wiklund U, Kamble PG & Eriksson JW. Time course of metabolic, neuroendocrine, and adipose effects during 2 years of follow-up after gastric bypass in patients with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 2021 **106** e4049–e4061. (https://doi.org/10.1210/clinem/dgab398)

10 Stefanidis A & Oldfield BJ. Neuroendocrine mechanisms underlying bariatric surgery: insights from human studies and animal models. *Journal of Neuroendocrinology* 2017 **29** e12534. (https://doi.org/10.1111/JNE.12534)

11 Sjöstrand M, Carlson K, Arnqvist HJ, Gudbjörnsdottir S, Landin-Olsson M, Lindmark S, Nyström L, Svensson MK, Eriksson JW & Bolinder J. Assessment of beta-cell function in young patients with type 2 diabetes: arginine-stimulated insulin secretion may reflect beta-cell reserve. *Journal of Internal Medicine* 2014 **275** 39–48. (https://doi.org/10.1111/JOIM.12116)

12 Ruetten H, Gebauer M, Raymond RH, Calle RA, Cobelli C, Ghosh A, Robertson RP, Shankar SS, Staten MA, Stefanovski D, *et al*. Mixed meal and intravenous L-arginine tests both stimulate incretin release across glucose tolerance in man: lack of correlation with β cell function. *Metabolic Syndrome and Related Disorders* 2018 **16** 406–415. (https://doi.org/10.1089/met.2018.0022)

13 Modvig IM, Kuhre RE, Jepsen SL, Xu SFS, Engelstoft MS, Egerod KL, Schwartz TW, Ørskov C, Rosenkilde MM & Holst JJ. Amino acids differ in their capacity to stimulate GLP-1 release from the perfused rat small intestine and stimulate secretion by different sensing mechanisms. *American Journal of Physiology: Endocrinology and Metabolism* 2021 **320** E874–E885. (https://doi.org/10.1152/AJPENDO.00026.2021)

14 Watkins JD, Koumanov F & Gonzalez JT. Protein- and calcium-mediated GLP-1 secretion: a narrative review. *Advances in Nutrition* 2021 **12** 2540–2552. (https://doi.org/10.1093/advances/nmab078)

15 Robertson RP, Bogachus LD, Oseid E, Parazzoli S, Patti ME, Rickels MR, Schuetz C, Dunn T, Pruett T, Balamurugan AN, *et al*. Assessment of β-cell mass and α- and β-cell survival and function by arginine stimulation in human autologous islet recipients. *Diabetes* 2015 **64** 565–572. (https://doi.org/10.2337/db14-0690)

16 Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, Cappa M, Bartolotta E, Dammacco F & Camanni F. Reliability of provocative

tests to assess growth hormone secretory status. Study in 472 normally growing children. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3323–3327. (https://doi.org/10.1210/jcem.81.9.8784091)
17 Bologna K, Cesana-Nigro N, Refardt J, Imber C, Vogt DR, Christ-Crain M & Winzeler B. Effect of arginine on the hypothalamic–pituitary–adrenal axis in individuals with and without vasopressin deficiency. *Journal of Clinical Endocrinology and Metabolism* 2020 **105** e2327–e2336. (https://doi.org/10.1210/clinem/dgaa157)
18 Georgia A, Asnis MCC, Febres G, Tsang A, Bessler M & Korner J. Roux-en-Y gastric bypass is associated with hyperinsulinemia but not increased maximal B-cell function. *Journal of the Endocrine Society* 2019 **3** 632–642. (https://doi.org/10.1210/js.2018-00213)
19 American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2020. *Diabetes Care* 2020 **43** S14–S31. (https://doi.org/10.2337/dc20-S002)
20 Katsogiannos P, Kamble PG, Boersma GJ, Karlsson FA, Lundkvist P, Sundbom M, Pereira MJ & Eriksson JW. Early changes in adipose tissue morphology, gene expression, and metabolism after RYGB in patients with obesity and T2D. *Journal of Clinical Endocrinology and Metabolism* 2019 **104** 2601–2613. (https://doi.org/10.1210/jc.2018-02165)
21 Simultaneous protocol for Mercodia Glucagon ELISA Mercodia. (10-1271-01). Technical Note, No: 34-161, v1.0. Uppsala, Sweden: Mercodia, 2019. (available at: https://cms.mercodia.com/wp-content/uploads/2019/06/tn34-161-simultaneous-protocol-for-glucagon-elisa-v1.pdf)
22 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. *Surgery for Obesity and Related Diseases* 2018 **14** 562–568. (https://doi.org/10.1016/j.soard.2018.01.039)
23 Purnell JQ, Johnson GS, Wahed AS, Dalla Man C, Piccinini F, Cobelli C, Prigeon RL, Goodpaster BH, Kelley DE, Staten MA, *et al*. Prospective evaluation of insulin and incretin dynamics in obese adults with and without diabetes for 2 years after Roux-en-Y gastric bypass. *Diabetologia* 2018 **61** 1142–1154. (https://doi.org/10.1007/s00125-018-4553-y)
24 Wewer Albrechtsen NJ, Hartmann B, Veedfald S, Windeløv JA, Plamboeck A, Bojsen-Møller KN, Idorn T, Feldt-Rasmussen B, Knop FK, Vilsbøll T, *et al*. Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia* 2014 **57** 1919–1926. (https://doi.org/10.1007/s00125-014-3283-z)
25 Stanley TL, Levitsky LL, Grinspoon SK & Misra M. Effect of body mass index on peak growth hormone response to provocative testing in children with short stature. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 4875–4881. (https://doi.org/10.1210/JC.2009-1369)
26 Luque RM & Kineman RD. Impact of obesity on the growth hormone axis: evidence for a direct inhibitory effect of hyperinsulinemia on pituitary function. *Endocrinology* 2006 **147** 2754–2763. (https://doi.org/10.1210/EN.2005-1549)
27 Engström BE, Burman P, Holdstock C, Öhrvall M, Sundbom M & Karlsson FA. Effects of gastric bypass on the GH/IGF-I axis in severe obesity – and a comparison with GH deficiency. *European Journal of Endocrinology* 2006 **154** 53–59. (https://doi.org/10.1530/eje.1.02069)
28 Devesa J. The complex world of regulation of pituitary growth hormone secretion: the role of ghrelin, klotho, and nesfatins in it. *Frontiers in Endocrinology* 2021 **12** 183. (https://doi.org/10.3389/FENDO.2021.636403/BIBTEX)
29 Xu HC, Pang YC, Chen JW, Cao JY, Sheng Z, Yuan JH, Wang R, Zhang CS, Wang LX & Dong J. Systematic review and meta-analysis of the change in ghrelin levels after Roux-en-Y gastric bypass. *Obesity Surgery* 2019 **29** 1343–1351. (https://doi.org/10.1007/s11695-018-03686-3)
30 Alba-Roth J, Müller OA, Schopohl J & von K. Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. *Journal of Clinical Endocrinology and Metabolism* 1988 **67** 1186–1189. (https://doi.org/10.1210/jcem-67-6-1186)
31 Corneli G, Somma C di, Baldelli R, Rovere S, Gasco V, Croce CG, Grottoli S, Maccario M, Colao A, Lombardi G, *et al*. The cut-off limits of the GH response to GH-releasing hormone-arginine test related to body mass index. *European Journal of Endocrinology* 2005 **153** 257–264. (https://doi.org/10.1530/eje.1.01967)
32 Lightman SL, Birnie MT & Conway-Campbell BL. Dynamics of ACTH and cortisol secretion and implications for disease. *Endocrine Reviews* 2020 **41** 470–490. (https://doi.org/10.1210/ENDREV/BNAA002)
33 Maheshwari HG, Pezzoli SS, Rahim A, Shalet SM, Thorner MO & Baumann G. Pulsatile growth hormone secretion persists in genetic growth hormone-releasing hormone resistance. *American Journal of Physiology: Endocrinology and Metabolism* 2002 **282** E943–E951. (https://doi.org/10.1152/ajpendo.00537.2001)
34 Cakir M, Sari R, Tosun O & Karayalcin U. Cortisol levels during an oral glucose tolerance test in lean and obese women. *Endocrine Research* 2005 **31** 213–218. (https://doi.org/10.1080/07435800500373199)
35 Fernández-Real JM, Ricart W & Casamitjana R. Lower cortisol levels after oral glucose in subjects with insulin resistance and abdominal obesity. *Clinical Endocrinology* 1997 **47** 583–588. (https://doi.org/10.1046/J.1365-2265.1997.3351120.X)
36 Reynolds RM, Walker BR, Syddall HE, Whorwood CB, Wood PJ & Phillips DIW. Elevated plasma cortisol in glucose-intolerant men: differences in responses to glucose and habituation to venepuncture. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1149–1153. (https://doi.org/10.1210/JCEM.86.3.7300)
37 Diz-Chaves Y, Gil-Lozano M, Toba L, Fandiño J, Ogando H, González-Matías LC & Mallo F. Stressing diabetes? The hidden links between insulinotropic peptides and the HPA axis. *Journal of Endocrinology* 2016 **230** R77–R94. (https://doi.org/10.1530/JOE-16-0118)
38 Tamboli RA, Antoun J, Sidani RM, Clements A, Harmata EE, Marks-Shulman P, Gaylinn BD, Williams B, Clements RH, Albaugh VL, *et al*. Metabolic responses to exogenous ghrelin in obesity and early after Roux-en-Y gastric bypass in humans. *Diabetes, Obesity and Metabolism* 2017 **19** 1267–1275. (https://doi.org/10.1111/dom.12952)
39 Tritos NA, Mun E, Bertkau A, Grayson R, Maratos-Flier E & Goldfine A. Serum ghrelin levels in response to glucose load in obese subjects post-gastric bypass surgery. *Obesity Research* 2003 **11** 919–924. (https://doi.org/10.1038/oby.2003.126)
40 Borg CM, Roux CW le, Ghatei MA, Bloom SR, Patel AG & Aylwin SJB. Progressive rise in gut hormone levels after Roux-en-Y gastric bypass suggests gut adaptation and explains altered satiety. *British Journal of Surgery* 2006 **93** 210–215. (https://doi.org/10.1002/BJS.5227)
41 Samat A, Malin SK, Huang H, Schauer PR, Kirwan JP & Kashyap SR. Ghrelin suppression is associated with weight loss and insulin action following gastric bypass surgery at 12 months in obese adults with type 2 diabetes. *Diabetes, Obesity and Metabolism* 2013 **15** 963–966. (https://doi.org/10.1111/DOM.12118)
42 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. *Obesity Surgery* 2011 **21** 896–901. (https://doi.org/10.1007/s11695-011-0412-3)
43 Jirapinyo P, Jin DX, Qazi T, Mishra N & Thompson CC. A meta-analysis of GLP-1 after Roux-en-Y gastric bypass: impact of surgical technique and measurement strategy. *Obesity Surgery* 2018 **28** 615–626. (https://doi.org/10.1007/s11695-017-2913-1)
44 Orskov C, Jeppesen J, Madsbad S & Holst JJ. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *Journal of Clinical Investigation* 1991 **87** 415–423. (https://doi.org/10.1172/JCI115012)
45 Svendsen B, Pedersen J, Albrechtsen NJW, Hartmann B, Toräng S, Rehfeld JF, Poulsen SS & Holst JJ. An analysis of cosecretion and coexpression of gut hormones from male rat proximal and distal small intestine. *Endocrinology* 2015 **156** 847–857. (https://doi.org/10.1210/EN.2014-1710)

46 Habib AM, Richards P, Rogers GJ, Reimann F & Gribble FM. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. *Diabetologia* 2013 **56** 1413–1416. (https://doi.org/10.1007/S00125-013-2887-Z)

47 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. (https://doi.org/10.1016/J.PEPTIDES.2015.08.013)

48 Jensen CZ, Bojsen-Møller KN, Svane MS, Holst LM, Hermansen K, Hartmann B, Albrechtsen NJW, Kuhre RE, Kristiansen VB, Rehfeld JF, *et al*. Responses of gut and pancreatic hormones, bile acids, and fibroblast growth factor-21 differ to glucose, protein, and fat ingestion after gastric bypass surgery. *American Journal of Physiology: Gastrointestinal and Liver Physiology* 2020 **318** G661–G672. (https://doi.org/10.1152/ajpgi.00265.2019)

49 Dirksen C, Bojsen-Møller KN, Jørgensen NB, Jacobsen SH, Kristiansen VB, Naver LS, Hansen DL, Worm D, Holst JJ & Madsbad S. Exaggerated release and preserved insulinotropic action of glucagon-like peptide-1 underlie insulin hypersecretion in glucose-tolerant individuals after Roux-en-Y gastric bypass. *Diabetologia* 2013 **56** 2679–2687. (https://doi.org/10.1007/s00125-013-3055-1)

50 Kashyap SR, Daud S, Kelly KR, Gastaldelli A, Win H, Brethauer S, Kirwan JP & Schauer PR. Acute effects of gastric bypass versus gastric restrictive surgery on beta-cell function and insulinotropic hormones in severely obese patients with type 2 diabetes. *International Journal of Obesity* 2010 **34** 462–471. (https://doi.org/10.1038/IJO.2009.254)

51 Ramracheya RD, McCulloch LJ, Clark A, Wiggins D, Johannessen H, Olsen MK, Cai X, Zhao CM, Chen D & Rorsman P. PYY-dependent restoration of impaired insulin and glucagon secretion in type 2 diabetes following Roux-en-Y gastric bypass surgery. *Cell Reports* 2016 **15** 944–950. (https://doi.org/10.1016/J.CELREP.2016.03.091)

52 Cummings DE, Overduin J & Foster-Schubert KE. Gastric bypass for obesity: mechanisms of weight loss and diabetes resolution. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2608–2615. (https://doi.org/10.1210/JC.2004-0433)

53 Honzawa N, Fujimoto K & Kitamura T. Cell autonomous dysfunction and insulin resistance in pancreatic α cells. *International Journal of Molecular Sciences* 2019 **20** 3699. (https://doi.org/10.3390/ijms20153699)

54 Ahrén B. Incretin dysfunction in type 2 diabetes: clinical impact and future perspectives. *Diabetes and Metabolism* 2013 **39** 195–201. (https://doi.org/10.1016/j.diabet.2013.03.001)

55 Holst JJ, Albrechtsen NJW, Pedersen J & Knop FKG. Glucagon and amino acids are linked in a mutual feedback cycle: the liver-α-cell axis. *Diabetes* 2017 **66** 235–240. (https://doi.org/10.2337/DB16-0994)

56 Eickhoff H, Louro T, Matafome P, Seiça R & Castro e Sousa F. Glucagon secretion after metabolic surgery in diabetic rodents. *Journal of Endocrinology* 2014 **223** 255–265. (https://doi.org/10.1530/JOE-14-0445)

57 Knop FK. Resolution of type 2 diabetes following gastric bypass surgery: involvement of gut-derived glucagon and glucagonotropic signalling? *Diabetologia* 2009 **52** 2270–2276. (https://doi.org/10.1007/s00125-009-1511-8)

58 Holst JJ, Bolette Hartmann CFD & Pedersen J. GLP 1/2, enteroglucagon, glicentin, and oxyntomodulin. In *Handbook of Biologically Active Peptides*, pp. 1241–1250. Amsterdam, Netherlands: Elsevier Inc., 2013. (https://doi.org/10.1016/B978-0-12-385095-9.00168-8)

Received in final form 13 June 2022
Accepted 27 June 2022
Accepted Manuscript published online 28 June 2022