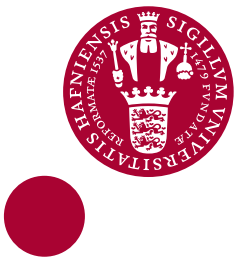

Augmented GLP-1 Secretion as Seen After Gastric Bypass May Be Obtained by Delaying Carbohydrate Digestion

Martinussen, Christoffer; Bojsen-Møller, Kirstine Nyvold; Dirksen, Carsten; Svane, Maria Saur; Kristiansen, Viggo Bjerregaard; Hartmann, Bolette; Holst, Jens Juul; Madsbad, Sten

Published in:
Journal of Clinical Endocrinology and Metabolism

DOI:
10.1210/jc.2018-02661

Publication date:
2019

Document version
Peer reviewed version

Document license:
Unspecified

Citation for published version (APA):
Martinussen, C., Bojsen-Møller, K. N., Dirksen, C., Svane, M. S., Kristiansen, V. B., Hartmann, B., ... Madsbad, S. (2019). Augmented GLP-1 Secretion as Seen After Gastric Bypass May Be Obtained by Delaying Carbohydrate Digestion. *Journal of Clinical Endocrinology and Metabolism*, *104*(8), 3233–3244. https://doi.org/10.1210/jc.2018-02661

Augmented GLP-1 secretion as seen after gastric bypass may be obtained by delaying carbohydrate digestion

Martinussen C, Bojsen-Møller KN, Dirksen C, Svane MS, Kristiansen VB, Hartmann B, Holst JJ, Madsbad S

The Journal of Clinical Endocrinology & Metabolism
Endocrine Society

Submitted: December 11, 2018
Accepted: March 01, 2019
First Online: March 07, 2019

Gut hormone secretion in response to carbohydrates

Augmented GLP-1 secretion as seen after gastric bypass may be obtained by delaying carbohydrate digestion

Martinussen C[1,2,3], Bojsen-Møller KN[1,3], Dirksen C[1,3], Svane MS[1,3], Kristiansen VB[4], Hartmann B[3], Holst JJ[3], Madsbad S[1,3]

[1]*Dept. of Endocrinology, Hvidovre Hospital, Denmark*

[2]*Danish Diabetes Academy, Odense University Hospital, Odense, Denmark*

[3]*Novo Nordisk Foundation Center for Basic Metabolic Research and Dept. of Biomedical Sciences, University of Copenhagen, Denmark*

[4]*Dept. of Surgical Gastroenterology, Hvidovre Hospital, Denmark*

ORCiD numbers:

0000-0002-7210-0807

Martinussen

Christoffer

Received 11 December 2018. Accepted 01 March 2019.

ClinicalTrials.gov registration number: NCT02879955

Context: Exaggerated postprandial Glucagon-like Peptide-1 (GLP-1) secretion seems important for weight loss and diabetes remission after Roux-en-Y gastric bypass (RYGB) and may result from carbohydrate absorption in the distal small intestine.
Objective: To investigate distal (GLP-1; Peptide YY, PYY) and proximal (Glucose-dependent Insulinotropic Polypeptide, GIP) gut hormone secretion in response to carbohydrates hydrolyzed at different rates. We hypothesized that slow digestion restricts proximal absorption, facilitating distal delivery of carbohydrates and thereby enhanced GLP-1 secretion in unoperated individuals, while this may not apply after RYGB.
Design: Single-blinded, randomized, crossover study.
Setting: Hvidovre Hospital, Denmark.
Participants: 10 RYGB- and 10 unoperated matched subjects.
Interventions: 4 separate days with ingestion of different carbohydrate loads, either rapidly/proximally digested (glucose+fructose; sucrose) or slowly/distally digested (isomaltulose; sucrose+acarbose).
Main outcome measures: GLP-1 secretion (area-under-the-curve above baseline). Secondary outcomes included PYY and GIP.
Results: Isomaltulose enhanced secretion of GLP-1 nearly 3-fold (p=0.02) and PYY 9-fold (p=0.08) compared with sucrose in unoperated subjects but had modest effect after RYGB. Acarbose failed to increase sucrose induced GLP-1 secretion in unoperated subjects and diminished the responses by 50% after RYGB (p=0.03). In both groups, GIP secretion was reduced by isomaltulose and even more so by sucrose+acarbose when compared to sucrose intake.
Conclusions: GLP-1 secretion depends on the rate of carbohydrate digestion, but in a different manner after RYGB. Enhanced GLP-1 secretion is central after RYGB, but it may also be obtained in unoperated individuals by delaying hydrolysis of carbohydrates, pushing their digestion and absorption distally in the small intestine.

GLP-1 responses to dietary carbohydrates were exaggerated after gastric bypass. Prolonged digestion led to greatly enhanced GLP-1 secretion in unoperated subjects, but less so after gastric bypass.

Introduction

Roux-en-Y gastric bypass (RYGB) surgery leads to substantial weight loss in morbidly obese patients and has profound effects on type 2 diabetes, resulting in improvements in glycemic control that are superior to those of conventional medical treatments (1,2). Exaggerated postprandial release of the L-cell hormones glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) appears to contribute importantly to improved glucose metabolism (GLP-1) and appetite control (GLP-1 + PYY) postoperatively (3–7). The exact mechanisms behind the exaggerated gut hormone release are incompletely understood, but dietary carbohydrates, as compared to fat and protein, seem to be particularly powerful in stimulating L-cell secretion after the surgery (8,9). The enhanced GLP-1 secretion after RYGB is thought to arise from the delivery of ingested carbohydrates to the distal intestine (10,11), where L-cell density is high (12–14). In addition, the accelerated glucose absorption after the surgery may play a major role since enteroendocrine cells appear to respond to the glucose absorption rate rather than to the presence of glucose in the intestinal lumen (15–18). Accordingly, reestablishing a normal rate of nutrient entry to the intestine after RYGB either by slowly infusing glucose through a tube inserted in the so-called *alimentary limb* or by administering the food into the bypassed gastric remnant, completely abolishes the postoperative increase in GLP-1 and insulin secretion (11,19,20). The importance of the intestinal site of nutrient delivery is further supported by studies in humans and rodents with diabetes, where an increased postprandial GLP-1 release accompanied by improved glucose tolerance is seen after surgical transposition of an ileum segment to the proximal intestine (21–24). Based on these findings, individuals with intact gastrointestinal anatomy could theoretically achieve significantly enhanced L-cell secretion by delivery of nutrients to the distal small intestine, an approach that might be exploited for the treatment of both diabetes and obesity.

Most dietary carbohydrates need to be digested to monosaccharides before absorption. The disaccharides sucrose and isomaltulose are hydrolyzed to the monosaccharides glucose and fructose by alpha-glucosidase enzymes in the brush border of the small intestine. While the alpha-1,2 glycosidic bond in sucrose is rapidly hydrolyzed, the alfa-1,6 linkage in isomaltulose is more resistant towards enzymatic breakdown (25). Consequently, isomaltulose is digested at a lower rate than sucrose resulting in a prolonged and presumably more distal absorption, which may affect GLP-1 secretion (26–28). The enzymatic activity of alpha-glucosidases can be inhibited pharmacologically by administration of acarbose. Acarbose ingested with sucrose has been found to increase GLP-1 release in unoperated individuals (29–31), while there is little effect when the drug is administered with a mixed meal (32–36), perhaps because the effect is overshadowed by the stimulatory effects of the other nutrients. Taken together, when digestion of carbohydrates is delayed, proximal absorption is assumed to be inhibited, leading to a more distal digestion and absorption and thereby greater release of GLP-1.

The primary objective of the present study was to investigate GLP-1 responses to oral loads of glucose and fructose, ingested either as separate monosaccharides or in the form of the disaccharides sucrose or isomaltulose in RYGB patients and unoperated individuals; and additionally, to investigate the effect of alpha-glucosidase inhibition by acarbose upon sucrose ingestion. We hypothesized that delayed digestion after isomaltulose as well as sucrose + acarbose would lead to distal absorption and thereby enhanced GLP-1 secretion in unoperated individuals, while a similar effect may not be seen after RYGB, where all carbohydrate loads are delivered to the distal intestine due to the surgical rearrangement.

Materials and methods

Participants and ethics

RYGB participants were eligible if they had uncomplicated laparoscopic RYGB surgery >1 year before the study. Exclusion criteria were current HbA1c>48 mmol/mol (>6.5 %) or a history of diabetes, hemoglobin <6.5 mmol/l, pregnancy or breast feeding, and co-morbidities or medications affecting glucose homeostasis or gastrointestinal function. Unoperated control subjects (CON) were matched for sex, age and BMI and fulfilled the same exclusion criteria with the addition of prior complicated surgery to the upper abdomen. Written informed consent was obtained from all participants before inclusion. Prior to initiation of the study, approval was obtained from the Regional Ethics Committee of the Capital Region of Denmark and from the Danish Data Protection Agency and registration was performed at ClinicalTrials.gov (NCT02879955). The study was conducted in accordance with the Helsinki declaration.

Study design

Participants were investigated on 4 experimental days separated by at least 2 days using different oral loads of mono- or disaccharides (provided by BENEO GmbH, Mannheim, Germany) dissolved in 200 mL water: a. 25 g glucose + 25 g fructose, b. 47.5 g sucrose, c. 47.5 g isomaltulose and d. 47.5 g sucrose combined with 100 mg acarbose (net contents, not including the content of water and impurities in the powders). Each of these loads provides ~0.14 mol glucose and fructose. One g of crushed paracetamol (Pamol; Nycomed, Roskilde, Denmark) was added to the solutions to allow assessment of paracetamol absorption as a measure of intestinal entry of the saccharide loads (gastric/pouch emptying). Study days were performed in a randomized order except the sucrose + acarbose day, which was performed last to avoid any carry-over effect of alpha-glucosidase inhibition on subsequent study days. The three other study days were randomized; 10 doublets of papers, representing different order of the test days, were drawn from an envelope, ensuring identical combinations of test days in the two groups.

Experimental day

Participants met after an overnight fast of 10-12 hours, were weighed and placed in a hospital bed at a 45 degrees reclined position throughout the test day, allowing no strenuous activity. A catheter was inserted in an antecubital vein for blood sampling. After three fasting samples, participants ingested 25 ml of water, to which was added, on the last day, 100 mg crushed acarbose (Glucobay, Bayer Pharma AG, Germany), and 5 min after, a steady ingestion over 10 min of the carbohydrate load was started. Blood was sampled frequently for 4 h postprandially (at times 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240 min). In addition, blood pressure measurements and visual analogue scores for satiety, hunger, nausea and stomach pain were carried out before and after (at times 30, 60, 90, 120, 180, 240 min) the carbohydrate load.

Sample collection and laboratory analyses

Blood was collected into prechilled EDTA tubes containing a DPP-4 inhibitor (valine-pyrrolidide, final concentration of 0.01 mM; a gift from Novo Nordisk, Bagsværd, Denmark) for analysis of GLP-1, GIP, PYY and glucagon; into EDTA tubes (or EDTA-Eppendorf tubes) for analysis of glucose and paracetamol; and into clot activator tubes for C-peptide measurement. Clot activator tubes were left to coagulate for 30 min (serum) and EDTA tubes were placed on ice and centrifuged without delay at 4°C. EDTA-Eppendorf tubes were centrifuged immediately and plasma for analysis of P-glucose was stored at 5°C before analysis at the end of the test day using YSI model 2300 STAT plus (YSI, Yellow Springs, OH). Samples for analysis of GLP-1, GIP, PYY, glucagon and paracetamol were stored at -20°C and serum at -80°C until batch analysis. Concentrations of C-peptide were determined

by Immulite 2000 analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA), paracetamol by enzyme immunoassay (Roche/Hitachi cobas c system) and total GLP-1, total GIP and glucagon as previously described (37); total hormone concentrations comprise intact hormone + primary DPP-4 metabolite. Determination of total PYY in plasma was carried out by RIA using a monoclonal antibody MAB8500 as described elsewhere (38).

Calculations and statistical analyses

Mean fasting concentrations were calculated as the mean of two fasting samples (three for glucose and C-peptide). Area-under-the-curve (AUC) was calculated using the trapezoidal rule and positive incremental AUC (piAUC) as the AUC above fasting concentrations for evaluation of postprandial secretory responses. Missing blood values (<1%) were imputed. HOMA2-IR was calculated based on triple measurement of glucose and C-peptide in fasting using the HOMA2 model (www.dtu.ox.ac.uk/homacalculator) (39). Wilcoxon signed rank test was used for within-group comparisons of the responses to the different sugar loads with sucrose as reference, and Mann-Whitney test was used for between-group comparisons of RYGB vs. CON. The function "wilcox.exact" from the package "exactRankTests" was applied to compute exact p-values using the Shift-Algorithm by Streitberg & Röhmel, which also corrects for ties. Effect size was quantified as the relative/percentage difference between arithmetic means. The GLP-1 response to the different carbohydrates was the primary endpoint, while all other comparisons were explorative secondary endpoints. Differences are evaluated from p-values after Bonferroni adjustment for multiple comparisons. Thus, p-values have been multiplied by 3 for within-group comparisons and by 4 for between-group comparisons of corresponding test days. An adjusted p-value <0.05 was considered significant. Statistical analysis was performed in R version 3.2.0 (www.R-project.org). Data are expressed as mean ± SEM unless otherwise stated.

Results

Characteristics of participants

Ten RYGB and ten non-surgical participants matched on sex, age and BMI were included. The groups had similar HbA1c, whereas insulin resistance assessed by HOMA2 was significantly lower after RYGB (**table 1**). RYGB patients were examined at a median of 3.9 (range: 2.2-5.8) years after surgery.

Fasting concentrations

Compared with CON, the RYGB group had significantly lower fasting plasma glucose (5.1 vs. 5.5 mmol/l, p<0.01) and serum C-peptide (524 vs. 833 pmol/l, p=0.02) concentration, while fasting plasma concentrations of gut hormones and glucagon did not differ significantly between groups (**table 1**). Generally, fasting plasma hormone concentrations did not differ substantially between study days (**table 2**), but differences did however reach significance for sucrose + acarbose compared with sucrose in both the RYGB (GLP-1, GIP, glucagon) and CON group (GIP).

Postprandial responses

Paracetamol absorption and glycemic excursions

Paracetamol absorption (as measure of gastric/pouch emptying) was very rapid after RYGB with higher peak values of plasma paracetamol occurring markedly earlier than in CON, while no within-group differences between study days were observed (**table 2, figure 1**). Glycemic excursions in response to the four different test loads generally reflected the expected digestibility of the sugars with the rapidly absorbed saccharides causing greater peak (glucose + fructose > sucrose > isomaltulose > sucrose + acarbose) and lower nadir of glucose concentration than the slowly absorbed saccharides in both groups although not all

comparisons reached significance (**table 2, figure 1**). RYGB operated participants were characterized by significantly higher peak plasma glucose compared with CON, but only in response to glucose + fructose and sucrose (adjusted p<0.01 for both), whereas peaks were comparable between groups after ingestion of isomaltulose or sucrose + acarbose (**table 2, figure 1**).

GLP-1 (primary outcome)

Intake of the slowly digested isomaltulose led to prolonged GLP-1 secretion in both groups. Consequently, isomaltulose enhanced GLP-1 secretion compared with the rapidly absorbed saccharides. In the CON group, isomaltulose ingestion resulted in 170% greater piAUC of GLP-1 (adjusted p=0.02 vs. sucrose) and higher peak concentrations (adjusted p=0.01 vs. sucrose) (**table 2, figure 2 and 3)**, whereas the relative effect after RYGB was much smaller and not significant (piAUC +50 %, adjusted p=0.32 vs. sucrose) (**table 2, figure 2 and 3**). The GLP-1 responses to sucrose (piAUC) were comparable to the responses induced by glucose + fructose in both RYGB (adjusted p=1.00) and CON subjects (adjusted p=1.00) (**figure 2 and 3**).

The GLP-1 response elicited by sucrose was diminished by acarbose in RYGB patients, leading to a 50% reduction in piAUC (adjusted p=0.03) and seemingly 30% lower peak levels (adjusted p=0.11), whereas these parameters were not affected by acarbose in CON (**table 2, figure 2 and 3**).

When compared with CON, the RYGB group was characterized by 3-fold greater overall GLP-1 secretion (piAUC) in response to glucose + fructose and sucrose (adjusted p<0.01 vs. CON for both), but not in response to isomaltulose or sucrose + acarbose (**figure 2 and 3**). Interestingly, the overall GLP-1 responses (piAUC) to isomaltulose in CON (1288 ± 243 min·pmol/l) were rather similar in magnitude to the responses induced by sucrose (1358 ± 182 min·pmol/l) and glucose + fructose (1319 ± 164 min·pmol/l) in RYGB operated patients (**figure 3**). However, the secretory profiles were markedly different between the RYGB and CON group for all comparisons with RYGB operated patients reaching much higher peak levels of GLP-1 for all comparisons (**table 2, figure 2**).

PYY, GIP, C-peptide and glucagon

Isomaltulose also appeared superior to sucrose in stimulating secretion of the other L-cell hormone PYY, but despite 9-fold greater piAUC in CON and a 2-fold increase after RYGB, significance was not reached (adjusted p=0.08 and 0.48, respectively) (**figure 2 and 3**). In contrast to the impact of alpha-glucosidase blockage on GLP-1, sucrose + acarbose increased piAUC of PYY 3-fold compared with sucrose alone but this was insignificant in both RYGB (adjusted p=0.25) and CON subjects (adjusted p=0.70) (**figure 2 and 3**). Secretion of PYY (piAUC) tended to be greater in RYGB operated individuals in response to the rapidly absorbed carbohydrates, sucrose (adjusted p=0.11 vs. CON) and glucose + fructose (adjusted p=0.12 vs. CON), but not in response to isomaltulose or sucrose + acarbose (adjusted p=1.00 and 0.87, respectively). (**figure 2 and 3**)**.**

Secretion of GIP was lower after isomaltulose compared with sucrose in both groups with a 50% reduction in piAUC and a 70% reduction in peak concentration after RYGB (adjusted p<0.01 vs. sucrose for both), while isomaltulose in CON decreased the peak concentration (adjusted p<0.01) and piAUC (adjusted p=0.06) of GIP by 30% compared with sucrose (**table 2, figure 2 and 3**). Intake of sucrose + acarbose resulted in 90% lower piAUC and 50% lower peak of GIP in both groups (adjusted p<0.01 vs. sucrose for all comparisons). Overall GIP secretion (piAUC) was comparable between the RYGB and CON group and the numerical differences in peak concentration were insignificant (glucose + fructose: adjusted p=0.99, sucrose: adjusted p=0.18, isomaltulose: adjusted p=1.00, sucrose + acarbose: adjusted p=0.11) (**table 2, figure 2 and 3**).

Alpha-glucosidase inhibition reduced piAUC and peak concentration of C-peptide by 40-50% in both groups (adjusted $p<0.01$ for sucrose + acarbose vs. sucrose in both RYGB and CON), while no between-group differences were observed (**table 2, figure 1**).

Glucagon concentration was suppressed by carbohydrate intake and total AUC of the hormone was comparable within and between groups (**table 2, figure 1**).

Discussion

In this study, we investigated GLP-1 secretion in RYGB operated patients and matched unoperated individuals in response to different oral loads of simple carbohydrates differing in digestibility. We assumed that slowly digested carbohydrates would bypass proximal absorption and reach more distal gut segments and therefore could be used as an experimental model to explore the importance of intestinal site of delivery for gut hormone secretion. The slowly digested disaccharide isomaltulose led to prolonged and greatly enhanced GLP-1 secretion (+170 % compared with sucrose) in unoperated individuals, while a relatively modest and statistically insignificant increase (+50 % compared with sucrose) was seen after RYGB where all ingested carbohydrates reach more distal parts of the small intestine. RYGB operated patients were characterized by 3-fold enhanced secretion of GLP-1 in response to the rapidly absorbed carbohydrates glucose + fructose and sucrose, while the difference between the RYGB and control group was less pronounced after intake of isomaltulose. Interestingly, the total secretory responses (piAUC) of GLP-1 after isomaltulose ingestion in unoperated individuals were comparable to the responses induced by sucrose or glucose + fructose in RYGB operated subjects, although the secretory profiles were markedly different. Surprisingly, retarding sucrose digestion by alpha-glucosidase inhibition (acarbose) neither enhanced GLP-1 secretion in unoperated subjects nor after RYGB. In fact, acarbose diminished the sucrose induced GLP-1 responses in the RYGB operated participants.

Glucose is known to stimulate GLP-1 secretion strongly while fructose elicits a modest response, particularly in the presence of glucose, in unoperated individuals (40,41). Exaggerated GLP-1 secretion in response to glucose intake is consistently reported after RYGB and has been linked to faster and more distal absorption of glucose after the surgery, as opposed to the situation in individuals with intact gastrointestinal anatomy where ingested glucose is absorbed exclusively in the proximal intestine with a lower density of L-cells. Enhanced GLP-1 responses to isomaltulose compared with sucrose have been reported by a few other studies in unoperated individuals (26–28) but have not previously been investigated after RYGB. The impact of isomaltulose on GLP-1 secretion may be explained by the slow hydrolysis of the alpha 1,6-bond allowing the glucose and fructose moieties to be absorbed in the distal intestine where L-cell density is highest. Accordingly, distal (jejunal/ileal) glucose infusion in humans has been demonstrated to increase GLP-1 secretion (42,43) and reduce food intake (43) when compared to duodenal infusion, although not consistently (44). In terms of this concept, isomaltulose would presumably not be expected to enhance GLP-1 secretion in RYGB operated patients, but this cannot be ruled out based on the present findings, challenging the hypothesis of distal delivery. However, it should be considered that sucrose absorption after RYGB occurs already in the alimentary limb, whereas isomaltulose is more slowly digested leading to a transfer to the distal small intestine with higher L-cell density, also in these patients. A rodent study comparing isomaltulose and sucrose demonstrated superiority of isomaltulose in stimulating GLP-1 secretion after oral ingestion, equality between the disaccharides after jejunal administration and greater responses to sucrose following ileal administration (45). Thus, it appears that the relationship between carbohydrate digestion rate and GLP-1 secretion depend on the site of delivery to the intestine. However, one must account for the possibility that the results of RYGB may not simply be derived from nutrient rerouting since gut hypertrophia and proliferation as well as

altered L-cell distribution and gene expression could also be involved (46,47). It is quite well established that GLP-1 secretion is closely linked to the rate of glucose absorption (15,18,48). However, the augmented GLP-1 secretion in response to isomaltulose is clearly not a result of accelerated glucose absorption since the glucose absorption rate was attenuated after isomaltulose ingestion (28). Nor is it likely that isomaltulose interacts directly with L-cells since the disaccharide has negligible impact on GLP-1 secretion in vitro (45).

Judging from the glycemic profiles, alpha-glucosidase inhibition by acarbose effectively suppressed sucrose digestion, but somewhat surprisingly, this did not lead to greater GLP-1 secretion in control subjects, again raising doubt about the idea that delaying glucose absorption to distal sites will enhance GLP-1 secretion. Previous studies in unoperated individuals have observed only slightly increased GLP-1 release when acarbose is ingested with sucrose (29,30), and generally no change when the drug is combined with a mixed meal (32–36). Given that absorption of glucose is necessary for GLP-1 secretion also in the ileum (18), the simplest explanation for the lack of effect is that the alpha glucosidase activity was inhibited so much that hydrolysis was partially prevented, leading to malabsorption and transfer of some of the sucrose load to the colon. This would be consistent with the rather flat glucose and C-peptide profiles. The effects of alpha-glucosidase inhibitors are probably influenced by several factors such as the timing of drug administration as well as the dosage and type of agent. Also, prolonged dosage once daily for a week appears to augment sucrose induced GLP-1 secretion to a much greater extent than single-dose administration in unoperated subjects (29). Acarbose has been used in the treatment of reactive hypoglycemia and the drug has previously been demonstrated to blunt postprandial GLP-1 and insulin hypersecretion in eight RYGB operated patients suffering from this condition (49). The same effect has also been observed in a case report of a patient suffering from reactive hypoglycemia after oesophagectomy (50). Thus, the inhibiting effect of acarbose on GLP-1 secretion after RYGB (and not in control subjects) may support that the rapid glucose absorption in the distal intestine is important for the enhanced GLP-1 secretion after the surgery as elegantly demonstrated previously by slowing intestinal glucose delivery in RYGB operated patients (11).

L-cells co-secrete GLP-1 and PYY in exaggerated amounts after RYGB, and there is evidence that these hormones act in synergy to reduce food intake after the surgery (7). PYY responses tended to be increased by isomaltulose compared with sucrose, and again the most robust effect was observed in unoperated individuals. In RYGB operated patients, the inhibition of GLP-1 secretion by acarbose was in sharp contrast to the effect of this drug on PYY, where secretion was clearly not inhibited. It has recently been established that the expression-pattern of L-cells varies considerably along the GI-tract with PYY expressed almost exclusively in the ileum and colon whereas GLP-1 also originates from the proximal intestine (14,18,51–53). In view of this, distal delivery of carbohydrates could augment secretion of PYY more than that of GLP-1. Thus, as discussed above, high dose acarbose administration after RYGB could lead to transfer of undigested sucrose to the colon and generation of short-chain fatty acids (SCFAs), which has been proposed to stimulate L-cell secretion through free fatty acid receptors GPR41 and GPR43 (54–59). The influence of colonic fermentation on gut hormone secretion in humans is incompletely understood, but it appears that SCFA may be particularly effective in stimulating the release of PYY (57,60,61). Thus, colonic transfer and fermentation of undigested sucrose might give rise to a stimulation of PYY release while leaving less sucrose behind in the small intestine to elicit GLP-1 secretion.

Contrary to L-cell secretion, responses of GIP were consistently lower after intake of the slowly digested saccharides in both groups. This agrees with previous findings in unoperated subjects (26–28,30,33) and was expected, considering the higher density of K-cells in the

proximal intestine (14). Overall GIP secretion (i.e. piAUC) was rather similar between the RYGB and control group despite seemingly different secretory profiles. Prospective studies have reported conflicting results regarding the effect of RYGB on postprandial GIP concentrations (62), but the changes are generally modest compared with the changes in L-cell secretion.

Increased meal-related glucagon levels are often reported after RYGB and have been proposed to arise from L-cell stimulation by nutrients in the distal intestine and/or stimulation of pancreatic secretion by fast absorption of amino acids caused by accelerated protein digestion (62,63). In our study, all carbohydrates suppressed glucagon secretion with no substantial differences between RYGB and control subjects, and glucagon was not enhanced by distal digestion, suggesting that other mechanisms besides co-secretion with GLP-1 from L-cells are involved (64). Secretory responses of C-peptide were considerably lowered by acarbose in both RYGB and control subjects in agreement with previous reports (30,32,33,49). Reduced beta-cell stimulation by glucose and incretin hormones following alpha-glucosidase inhibition may explain this finding.

Increased hepatic and peripheral insulin sensitivity are well-established responses to negative energy balance and weight loss and the improvements in these parameters after RYGB therefore come as no surprise (65). However, HOMA2-IR was lower after RYGB than in the matched controls, although our RYGB operated patients were examined in the weight stable phase approximately 4 years after surgery. Thus, the higher insulin sensitivity after the surgery may to some extent be independent of BMI as well as negative energy balance. Improvements in insulin sensitivity after RYGB beyond than what can be predicted from weight loss have been described previously (66).

In conclusion, gastric bypass operated patients are characterized by augmented GLP-1 secretion in response to rapidly absorbed carbohydrates, consistent with fast delivery to and absorption in distal segments of the gut. Accordingly, enhanced secretion of GLP-1 could also be obtained in matched unoperated subjects in response to the slowly and therefore more distally digested disaccharide isomaltulose, which had a modest and statistically insignificant effect in gastric bypass operated patients. By contrast, retarding sucrose digestion using the alpha-glucosidase inhibitor acarbose failed to enhance GLP-1 secretion in unoperated individuals, raising doubt about the importance of distal delivery, while acarbose diminished GLP-1 responses after gastric bypass. Potent inhibition of sucrose hydrolysis and possibly malabsorption caused by the large dose of acarbose employed might explain this. Elucidating the digestive and absorptive processes implicated in gut hormone secretion after gastric bypass may aid the discovery of nutritional or pharmacological modalities mimicking the effects of the surgery.

the Danish Diabetes Academy funded by the Novo Nordisk Foundation, Christoffer Martinussen; H2020 European Research Council http://dx.doi.org/10.13039/100010663, 695069-BYPASSWITHOUTSURGERY, Jens Juul Holst; the Novo Nordisk Foundation, NNF15OC0017188, Carsten Dirksen; the Novo Nordisk Foundation, Sten Madsbad; the Research Foundation for health research of the Capital Region of Denmark, Sten Madsbad; the Internal Research Foundation at Hvidovre Hospital, Christoffer Martinussen

Acknowledgements

We greatly appreciate the technical assistance of Alis Sloth Andersen, Sussi Polmann, Fozia Zia and Jette Nymann (Hvidovre University Hospital, Copenhagen, Denmark) as well as Lene Brus Albæk (Department of Biomedical Sciences, University of Copenhagen, Denmark).

Correspondence to: Sten Madsbad, Dep. of Endocrinology, Hvidovre Hospital, Kettegaard Allé 30, 2650 Hvidovre, Denmark, Email: sten.madsbad@regionh.dk, Phone: +45 21 69 19 11

Disclosure summary:
This work was supported by grants from the Danish Diabetes Academy funded by the Novo Nordisk Foundation; the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 695069-BYPASSWITHOUTSURGERY); the Novo Nordisk Foundation (NNF15OC0017188); the Research Foundation for health research of the Capital Region of Denmark and the Internal Research Foundation at Hvidovre Hospital. CM, KNBM, CD, MSV, BH, JJH and SM are affiliated with the Novo Nordisk Foundation Center for Basic Metabolic Research funded by the Novo Nordisk Foundation. VBK has no disclosures.

References

1. **Sjöström L**. Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery. *J Intern Med*. 2013;273(3):219-234.
2. **Pories WJ, Swanson MS, MacDonald KG, Long SB, Morris PG, Brown BM, Barakat HA, deRamon RA, Israel G, Dolezal JM**. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Ann Surg*. 1995;222(3):339-50; discussion 350-2.
3. **Jørgensen NB, Dirksen C, Bojsen-Møller KN, Jacobsen SH, Worm D, Hansen DL, Kristiansen VB, Naver L, Madsbad S, Holst JJ**. Exaggerated glucagon-like peptide 1 response is important for improved β-cell function and glucose tolerance after Roux-en-Y gastric bypass in patients with type 2 diabetes. *Diabetes*. 2013;62(9):3044-3052.
4. **Dirksen C, Jørgensen NB, Bojsen-Møller KN, Kielgast U, Jacobsen SH, Clausen TR, Worm D, Hartmann B, Rehfeld JF, Damgaard M, Madsen JL, Madsbad S, Holst JJ, Hansen DL.** 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):1452-1459.
5. **le Roux CW, Welbourn R, Werling M, Osborne A, Kokkinos A, Laurenius A, Lönroth H, Fändriks L, Ghatei M a, Bloom SR, Olbers T.** Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann Surg*. 2007;246(5):780-785.
6. **de Hollanda A, Jiménez A, Corcelles R, Lacy AM, Patrascioiu I, Vidal J**. Gastrointestinal hormones and weight loss response after Roux-en-Y gastric bypass. *Surg Obes Relat Dis*. 2014;10(5):814-9.
7. **Svane MS, Jørgensen NB, Bojsen-Møller KN, Dirksen C, Nielsen S, Kristiansen VB, Toräng S, Wewer Albrechtsen NJ, Rehfeld JF, Hartmann B, Madsbad S, Holst JJ.** Peptide YY and glucagon-like peptide-1 contribute to decreased food intake after Roux-en-Y gastric bypass surgery. *Int J Obes (Lond)*. 2016;40(11):1699-1706.
8. **Jensen C, Bojsen-Møller K, Svane M, Holst L, Hermansen K, Holst J, Madsbad S**. Exaggerated postprandial GLP-1 secretion after Roux-en-Y gastric bypass is highest after glucose compared with fat and protein ingestion. *Program of the 52nd Annual Meeting of the European Association for the Study of Diabetes*, Munich, Germany, **2016,** *Abstr nr 530*.
9. **Jacobsen SH, Olesen SC, Dirksen C, Jørgensen NB, Bojsen-Møller KN, Kielgast U, Worm D, Almdal T, Naver LS, Hvolris LE, Rehfeld JF, Wulff BS, Clausen TR, Hansen DL, Holst JJ, Madsbad S.** Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects. *Obes Surg*. 2012;22(7):1084-1096.

10. **Jacobsen SH, Bojsen-Møller KN, Dirksen C, Jørgensen NB, Clausen TR, Wulff BS, Kristiansen VB, Worm D, Hansen DL, Holst JJ, van Hall G, Madsbad S.** Effects of gastric bypass surgery on glucose absorption and metabolism during a mixed meal in glucose-tolerant individuals. *Diabetologia*. 2013;56(10):2250-2254.
11. **Nguyen NQ, Debreceni TL, Bambrick JE, Bellon M, Wishart J, Standfield S, Rayner CK, Horowitz M**. Rapid gastric and intestinal transit is a major determinant of changes in blood glucose, intestinal hormones, glucose absorption and postprandial symptoms after gastric bypass. *Obesity*. 2014;22(9):2003-2009.
12. **Sjölund K, Sandén G, Håkanson R, Sundler F**. Endocrine cells in human intestine: an immunocytochemical study. *Gastroenterology*. 1983;85(5):1120-1130.
13. **Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B**. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest*. 1992;22(4):283-291.
14. **Jorsal T, Rhee NA, Pedersen J, Wahlgren CD, Mortensen B, Jepsen SL, Jelsing J, Dalbøge LS, Vilmann P, Hassan H, Hendel JW, Poulsen SS, Holst JJ, Vilsbøll T, Knop FK.** Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. *Diabetologia*. 2018;61(2):284-294.
15. **Holst JJ, Gribble F, Horowitz M, Rayner CK.** Roles of the Gut in Glucose Homeostasis. *Diabetes Care*. 2016;39(6):884-92.
16. **Schirra J, Katschinski M, Weidmann C, Schäfer T, Wank U, Arnold R, Göke B**. Gastric emptying and release of incretin hormones after glucose ingestion in humans. *J Clin Invest*. 1996;97(1):92-103.
17. **Little TJ, Doran S, Meyer JH, Smout AJPM, O'Donovan DG, Wu K-L, Jones KL, Wishart J, Rayner CK, Horowitz M, Feinle-Bisset C**. The release of GLP-1 and ghrelin, but not GIP and CCK, by glucose is dependent upon the length of small intestine exposed. *Am J Physiol Endocrinol Metab*. 2006;291(3):E647-55.
18. **Kuhre RE, Christiansen CB, Saltiel MY, Wewer Albrechtsen NJ, Holst JJ**. On the relationship between glucose absorption and glucose-stimulated secretion of GLP-1, neurotensin, and PYY from different intestinal segments in the rat. *Physiol Rep*. 2017;5(23):e13507.
19. **Dirksen C, Hansen DL, Madsbad S, Hvolris LE, Naver LS, Holst JJ, Worm D**. Postprandial diabetic glucose tolerance is normalized by gastric bypass feeding as opposed to gastric feeding and is associated with exaggerated GLP-1 secretion: a case report. *Diabetes Care*. 2010;33(2):375-377.
20. **Pournaras DJ, Aasheim ET, Bueter M, Ahmed AR, Welbourn R, Olbers T, le Roux CW**. Effect of bypassing the proximal gut on gut hormones involved with glycemic control and weight loss. *Surg Obes Relat Dis*. 2012;8(4):371-4.
21. **Grueneberger JM, Fritz T, Zhou C, Meyer S, Karcz-Socha I, Sawczyn T, Stygar D, Goos M, Hopt UT, Küsters S**. Long segment ileal transposition leads to early amelioration of glucose control in the diabetic obese Zucker rat. *Wideochir Inne Tech Maloinwazyjne.* 2013;8(2):130-138.
22. **Patriti A, Facchiano E, Annetti C, Aisa MC, Galli F, Fanelli C, Donini A**. Early improvement of glucose tolerance after ileal transposition in a non-obese type 2 diabetes rat model. *Obes Surg*. 2005;15(9):1258-1264.
23. **Kindel TL, Yoder SM, Seeley RJ, D'Alessio DA, Tso P**. Duodenal-jejunal exclusion improves glucose tolerance in the diabetic, Goto-Kakizaki rat by a GLP-1 receptor-mediated mechanism. *J Gastrointest Surg*. 2009;13(10):1762-1772.
24. **DePaula AL, Macedo AL V., Rassi N, Vencio S, Machado CA, Mota BR, Silva LQ, Halpern A, Schraibman V**. Laparoscopic treatment of metabolic syndrome in patients with type 2 diabetes mellitus. *Surg Endosc*. 2008;22(12):2670-2678.

25. **Holub I, Gostner A, Theis S, Nosek L, Kudlich T, Melcher R, Scheppach W**. Novel findings on the metabolic effects of the low glycaemic carbohydrate isomaltulose (Palatinose). *Br J Nutr*. 2010;103(12):1730-1737.
26. **Maeda A, Miyagawa JI, Miuchi M, Nagai E, Konishi K, Matsuo T, Tokuda M, Kusunoki Y, Ochi H, Murai K, Katsuno T, Hamaguchi T, Harano Y, Namba M.** Effects of the naturally-occurring disaccharides, palatinose and sucrose, on incretin secretion in healthy non-obese subjects. *J Diabetes Investig*. 2013;4(3):281-286.
27. **Keyhani-Nejad F, Kemper M, Schueler R, Pivovarova O, Rudovich N, Pfeiffer AFH**. Effects of Palatinose and Sucrose Intake on Glucose Metabolism and Incretin Secretion in Subjects With Type 2 Diabetes. *Diabetes Care*. 2016;39(3):e38-e39.
28. **Ang M, Linn T**. Comparison of the effects of slowly and rapidly absorbed carbohydrates on postprandial glucose metabolism in type 2 diabetes mellitus patients: a randomized trial. *Am J Clin Nutr*. 2014;100(4):1059-1068.
29. **Fukase N, Takahashi H, Manaka H, Igarashi M, Yamatani K, Daimon M, Sugiyama K, Tominaga M, Sasaki H**. Differences in glucagon-like peptide-1 and GIP responses following sucrose ingestion. *Diabetes Res Clin Pract*. 1992;15(3):187-195.
30. **Seifarth C, Bergmann J, Holst JJ, Ritzel R, Schmiegel W, Nauck MA**. Prolonged and enhanced secretion of glucagon-like peptide 1 (7-36 amide) after oral sucrose due to alpha-glucosidase inhibition (acarbose) in Type 2 diabetic patients. *Diabet Med*. 1998;15(6):485-491.
31. **Qualmann C, Nauck MA, Holst JJ, Orskov C, Creutzfeldt W**. Glucagon-like peptide 1 (7-36 amide) secretion in response to luminal sucrose from the upper and lower gut. A study using alpha-glucosidase inhibition (acarbose). *Scand J Gastroenterol*. 1995;30(9):892-896.
32. **Chen Z, Fu X, Kuang J, Chen J, Chen H, Pei J, Yang H**. Single-dose acarbose decreased glucose-dependent insulinotropic peptide and glucagon levels in Chinese patients with newly diagnosed type 2 diabetes mellitus after a mixed meal. *BMC Endocr Disord*. 2016;16(1):55.
33. **Hücking K, Kostic Z, Pox C, Ritzel R, Holst JJ, Schmiegel W, Nauck MA**. α-glucosidase inhibition (acarbose) fails to enhance secretion of glucagon-like peptide 1 (7-36 amide) and to delay gastric emptying in Type 2 diabetic patients. *Diabet Med*. 2005;22(4):470-476.
34. **Ueno H, Tsuchimochi W, Wang H-W, Yamashita E, Tsubouchi C, Nagamine K, Sakoda H, Nakazato M**. Effects of Miglitol, Acarbose, and Sitagliptin on Plasma Insulin and Gut Peptides in Type 2 Diabetes Mellitus: A Crossover Study. *Diabetes Ther*. 2015;6(2):187-196.
35. **DeLeon MJ, Chandurkar V, Albert SG, Mooradian AD**. Glucagon-like peptide-1 response to acarbose in elderly type 2 diabetic subjects. *Diabetes Res Clin Pract*. 2002;56(2):101-106.
36. **Enç FY, I˙meryüz N, Akin L, Turoğlu T, Dede F, Haklar G, Tekeşi˙n N, Beki˙roğlu N, Yeğen B, Rehfeld JF, Holst JJ, Ulusoy NB**. Inhibition of gastric emptying by acarbose is correlated with GLP-1 response and accompanied by CCK release. *Am J Physiol Liver Physiol*. 2001;281(3):G752-G763.
37. **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(12):2679-2687.
38. **Toräng S, Bojsen-Møller KN, Svane MS, Hartmann B, Rosenkilde MM, Madsbad S, Holst JJ**. In vivo and in vitro degradation of peptide YY3-36 to inactive peptide YY3-34 in humans. *Am J Physiol Regul Integr Comp Physiol*. 2016;310(9):R866-74.

39. **Wallace TM, Levy JC, Matthews DR**. Use and abuse of HOMA modeling. *Diabetes Care*. 2004;27(6):1487-1495.
40. **Kuhre RE, Gribble FM, Hartmann B, Reimann F, Windeløv JA, Rehfeld JF, Holst JJ**. Fructose stimulates GLP-1 but not GIP secretion in mice, rats, and humans. *Am J Physiol Liver Physiol*. 2014;306(7):G622-G630.
41. **Kong MF, Chapman I, Goble E, Wishart J, Wittert G, Morris H, Horowitz M**. Effects of oral fructose and glucose on plasma GLP-1 and appetite in normal subjects. *Peptides*. 1999;20(5):545-551.
42. **Wu T, Thazhath SS, Marathe CS, Bound MJ, Jones KL, Horowitz M, Rayner CK, Thazhath S, Marathe C, Bound M, Jones K, Rayner C**. Comparative effect of intraduodenal and intrajejunal glucose infusion on the gut-incretin axis response in healthy males. *Nutr Diabetes*. 2015;5(5):156-1.
43. **Poppitt SD, Shin HS, McGill AT, Budgett SC, Lo K, Pahl M, Duxfield J, Lane M, Ingram JR**. Duodenal and ileal glucose infusions differentially alter gastrointestinal peptides, appetite response, and food intake: A tube feeding study. *Am J Clin Nutr*. 2017;106(3):725-735.
44. **Chaikomin R, Wu K-L, Doran S, Meyer JH, Jones KL, Feinle-Bisset C, Horowitz M, Rayner CK**. Effects of mid-jejunal compared to duodenal glucose infusion on peptide hormone release and appetite in healthy men. *Regul Pept*. 2008;150(1-3):38-42.
45. **Hira T, Muramatsu M, Okuno M, Hara H**. GLP-1 secretion in response to oral and luminal palatinose (isomaltulose) in rats. *J Nutr Sci Vitaminol (Tokyo)*. 2011;57(1):30-35.
46. **Hansen CF, Bueter M, Theis N, Lutz T, Paulsen S, Dalbøge LS, Vrang N, Jelsing J**. Hypertrophy Dependent Doubling of L-Cells in Roux-en-Y Gastric Bypass Operated Rats. *PLoS One*. 2013;8(6).
47. **Rhee NA, Wahlgren CD, Pedersen J, Mortensen B, Langholz E, Wandall EP, Friis SU, Vilmann P, Paulsen SJ, Kristiansen VB, Jelsing J, Dalbøge LS, Poulsen SS, Holst JJ, Vilsbøll T, Knop FK.** Effect of Roux-en-Y gastric bypass on the distribution and hormone expression of small-intestinal enteroendocrine cells in obese patients with type 2 diabetes. *Diabetologia*. 2015;58(10):2254-2258.
48. **Gorboulev V, Schürmann A, Vallon V, Kipp H, Jaschke A, Klessen D, Friedrich A, Scherneck S, Rieg T, Cunard R, Veyhl-Wichmann M, Srinivasan A, Balen D, Breljak D, Rexhepaj R, Parker HE, Gribble FM, Reimann F, Lang F, Wiese S, Sabolic I, Sendtner M, Koepsell H.** Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. *Diabetes*. 2012;61(1):187-96.
49. **Valderas JP, Ahuad J, Rubio L, Escalona M, Pollak F, Maiz A**. Acarbose improves hypoglycaemia following gastric bypass surgery without increasing glucagon-like peptide 1 levels. *Obes Surg*. 2012;22(4):582-586.
50. **Imhof A, Schneemann M, Schaffner A, Brändle M**. Reactive hypoglycaemia due to late dumping syndrome: successful treatment with acarbose. *Swiss Med Wkly*. 2001;131(5-6):81-83.
51. **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(3):847-857.
52. **Egerod KL, Engelstoft MS, Grunddal K V., Nøhr MK, Secher A, Sakata I, Pedersen J, Windeløv JA, Füchtbauer EM, Olsen J, Sundler F, Christensen JP, Wierup N, Olsen J V., Holst JJ, Zigman JM, Poulsen SS, Schwartz TW.** A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY, and neurotensin but not somatostatin. *Endocrinology*. 2012;153(12):5782-5795.
53. **Habib AM, Richards P, Cairns LS, Rogers GJ, Bannon CAM, Parker HE, Morley TCE, Yeo GSH, Reimann F, Gribble FM**. Overlap of endocrine hormone

expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Endocrinology*. 2012;153(7):3054-3065.
54. **Tarini J, Wolever TMS**. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Appl Physiol Nutr Metab*. 2010;35(1):9-16.
55. **Freeland KR, Wilson C, Wolever TMS**. Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. *Br J Nutr*. 2010;103(1):82-90.
56. **Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM**. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes*. 2012;61(2):364-371.
57. **Larraufie P, Martin-Gallausiaux C, Lapaque N, Dore J, Gribble FM, Reimann F, Blottiere HM**. SCFAs strongly stimulate PYY production in human enteroendocrine cells. *Sci Rep*. 2018;8(1):1-9.
58. **Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, MacDougall K, Preston T, Tedford C, Finlayson GS, Blundell JE, Bell JD, Thomas EL, Mt-Isa S, Ashby D, Gibson GR, Kolida S, Dhillo WS, Bloom SR, Morley W, Clegg S, Frost G.** Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut*. 2015;64(11):1744-1754.
59. **Christiansen CB, Gabe MBN, Svendsen B, Dragsted LO, Rosenkilde MM, Holst JJ**. The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am J Physiol Liver Physiol*. 2018;315(1):G53-G65.
60. **van der Beek CM, Canfora EE, Lenaerts K, Troost FJ, Damink SWMO, Holst JJ, Masclee AAM, Dejong CHC, Blaak EE**. Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clin Sci*. 2016;130(22):2073-2082.
61. **Canfora EE, van der Beek CM, Jocken JWE, Goossens GH, Holst JJ, Olde Damink SWM, Lenaerts K, Dejong CHC, Blaak EE**. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci Rep*. 2017;7(1):2360.
62. **Dirksen C, Jørgensen NB, Bojsen-Møller KN, Jacobsen SH, Hansen DL, Worm D, Holst JJ, Madsbad S**. Mechanisms of improved glycaemic control after Roux-en-Y gastric bypass. *Diabetologia*. 2012;55(7):1890-1901.
63. **Bojsen-Møller KN, Jacobsen SH, Dirksen C, Jørgensen NB, Reitelseder S, Jensen JB, Kristiansen VB, Holst JJ, Hall G Van, Madsbad S.** Accelerated protein digestion and amino acid absorption after Roux-en-Y gastric bypass. *Am J Clin Nutr*. 2015;(C):1-8.
64. **Wewer Albrechtsen NJ, Hartmann B, Veedfald S, Windeløv JA, Plamboeck A, Bojsen-Møller KN, Idorn T, Feldt-Rasmussen B, Knop FK, Vilsbøll T, Madsbad S, Deacon CF, Holst JJ.** Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia*. 2014;57(9):1919-1926.
65. **Bojsen-Møller KN, Dirksen C, Jørgensen NB, Jacobsen SH, Serup AK, Albers PH, Hansen DL, Worm D, Naver L, Kristiansen VB, Wojtaszewski JFP, Kiens B, Holst JJ, Richter EA, Madsbad S.** Early enhancements of hepatic and later of peripheral insulin sensitivity combined with increased postprandial insulin secretion contribute to improved glycemic control after Roux-en-Y gastric bypass. *Diabetes*. 2014;63(5):1725-1737.
66. **Bikman BT, Zheng D, Pories WJ, Chapman W, Pender JR, Bowden RC, Reed MA, Cortright RN, Tapscott EB, Houmard JA, Tanner CJ, Lee J, Dohm GL.**

Mechanism for Improved Insulin Sensitivity after Gastric Bypass Surgery. 2008;93(12):4656-63.

Figure 1. Data are mean + SEM. Concentration of glucose, C-peptide, glucagon and paracetamol in response to intake of glu+fru (dotted black line), suc (solid black line, black circles), iso (solid gray line, gray squares) and suc+aca (dashed black line, white triangles) in Roux en-Y gastric bypass operated patients (RYGB) to the left and control subjects (CON) to the right. glu=glucose, fru=fructose, suc=sucrose, iso=isomaltulose, aca=acarbose.

Figure 2. Data are mean + SEM. Concentration of Glucagon-like Peptide-1 (GLP-1), Peptide YY (PYY), and Glucose-dependent Insulinotropic Polypeptide (GIP) in response to intake of glu+fru (dotted black line), suc (solid black line, black circles), iso (solid gray line, gray squares) and suc+aca (dashed black line, white triangles) in Roux en-Y gastric bypass operated patients (RYGB) to the left and control subjects (CON) to the right. glu=glucose, fru=fructose, suc=sucrose, iso=isomaltulose, aca=acarbose.

Figure 3. Data are mean + SEM. Secretory responses of Glucagon-like Peptide-1 (GLP-1), Peptide YY (PYY), and Glucose-dependent Insulinotropic Polypeptide (GIP) assessed as the positive incremental area-under-the-curve with Roux en-Y gastric bypass operated patients (RYGB) to the left and control subjects (CON) to the right. *adjusted p<0.05 **adjusted p<0.01 vs. suc (within-group comparison using Wilcoxon signed rank test followed by Bonferroni adjustment for multiple comparisons). §adjusted p<0.05 §§adjusted p<0.01 vs. RYGB (between-group comparison of corresponding test days using Mann-Whitney test followed by Bonferroni adjustment for multiple comparisons). glu=glucose, fru=fructose, suc=sucrose, iso=isomaltulose, aca=acarbose.

Table 1. Baseline characteristics and fasting concentrations for Roux en-Y gastric bypass (RYGB) and control (CON) participants.

	RYGB	CON	P
Number	10	10	
Gender (m/f)	5/5	5/5	
Age (years)	43.3 ± 1.8	42.7 ± 3.2	0.97
BMI (kg/m^2)	33.0 ± 1.7	32.5 ± 1.8	0.58
HbA1c (mmol/mol)	36 ± 0.9	37 ± 0.8	0.60
HOMA2-IR C-peptide	1.16 ± 0.08	1.89 ± 0.23	0.02
Fasting glucose (mmol/l)	5.1 ± 0.08	5.5 ± 0.08	<0.01
Fasting C-peptide (pmol/l)	524 ± 34	833 ± 103	0.02
Fasting glucagon (pmol/l)	6.4 ± 1.1	9.0 ± 1.8	0.11
Fasting GLP-1 (pmol/l)	7.3 ± 1.1	7.7 ± 0.9	0.52
Fasting PYY (pmol/l)	6.0 ± 0.8	5.7 ± 0.5	0.73
Fasting GIP (pmol/l)	6.4 ± 1.2	6.0 ± 1.3	0.67

Data are mean ± SEM. For calculation of fasting concentrations in each group, the mean of the four test days for each study participant was used. P-values for between-group comparison using Mann-Whitney test. BMI=Body Mass Index, HOMA2-IR C-peptide=homeostatic model assessment of insulin resistance based on C-peptide, GLP-1=Glucagon-like Peptide-1, PYY=Peptide YY, GIP=Glucose-dependent Insulinotropic Polypeptide.

Table 2. Postprandial responses to ingestion of simple carbohydrates in Roux en-Y gastric bypass (RYGB) and control (CON) participants.

	RYGB				CON			
	glu + fru	**suc**	**iso**	**suc + aca**	**glu + fru**	**suc**	**iso**	**suc + aca**
Number	10	10	10	10	10	10	10	10
Glucose								
fasting (mmol/l)	5.0 ± 0.09	5.1 ± 0.11	5.1 ± 0.12	5.0 ± 0.07	5.4 ± 0.11§	5.5 ± 0.09	5.5 ± 0.11	5.6 ± 0.09§§
max (mmol/l)	9.8 ± 0.38	9.3 ± 0.37	7.4 ± 0.36**	6.7 ± 0.23**	8.1 ± 0.21§§	7.7 ± 0.17§§	7.0 ± 0.20*	6.6 ± 0.13**

min (mmol/l)	4.1 ± 0.17	4.1 ± 0.21	4.4 ± 0.14	4.7 ± 0.08*	4.5 ± 0.07	4.6 ± 0.08	4.8 ± 0.12	5.0 ± 0.08**§
C-peptide								
fasting (pmol/l)	512 ± 25	509 ± 36	526 ± 40	549 ± 42	825 ± 111	839 ± 111	822 ± 93	846 ± 111
max (pmol/l)	2888 ± 155	2762 ± 402	2135 ± 382*	1341 ± 109**	2605 ± 277	2367 ± 220	2116 ± 217	1520 ± 141**
piAUC (min·nmol/l)	129 ± 8	123 ± 12	129 ± 19	63 ± 10**	125±12	119± 9	130 ± 15	68 ± 6**
Glucagon								
fasting (pmol/l)	6.5 ± 1.7	5.3 ± 1.1	5.2 ± 1.0	8.7 ± 1.2*	8.7 ± 1.9	9.4 ± 1.9	7.8 ± 1.5	10.3 ± 2.2
min (pmol/l)	2.3 ± 0.5	2.6 ± 0.7	2.8 ± 0.6	1.9 ± 0.3	4.7 ± 1.4	4.1 ± 1.2	3.9 ± 1.1	4.5± 1.1
tAUC (min·pmol/l)	1227 ± 176	1238 ± 207	1304 ± 216	1268 ± 136	1796 ± 394	1862 ± 451	1627 ± 316	1766 ± 351
Paracetamol								
max (µg/ml)	18 ± 1.3	18 ± 0.8	17 ± 1.2	19 ± 1.4	9.4 ± 0.5§§	9.6 ± 0.7§§	8.5 ± 0.4§§	9.1 ± 0.6§§
$time_{max}$ (min)	18 ±1.5	18 ± 1.5	17 ± 0.8	21 ± 4.4	63 ± 8.0§§	65 ± 7.4§§	76 ± 12.6§§	63 ± 9.2§§
GLP-1								
fasting (pmol/l)	5.8 ± 1.4	6.0 ± 0.9	6.1 ± 1.3	11.2 ± 1.6*	7.5 ± 1.0	7.1 ± 1.0	6.9 ± 0.9	9.3 ± 1.4
max (pmol/l)	49 ± 7.4	45 ± 7.0	41 ± 7.2	32 ± 4.3	18 ± 3.8§§	15 ± 1.3§§	20 ± 1.1*§§	16 ± 1.8§
PYY								
fasting (pmol/l)	6.6 ± 1.1	6.0 ± 0.9	5.0 ± 0.8	6.6 ± 1.2	5.8 ± 0.8	5.6 ± 0.7	3.9 ± 0.9	7.6 ± 1.1
max (pmol/l)	14 ± 0.9	12 ± 2.0	11 ± 1.2	14 ± 1.7	9.8 ± 1.3	7.9 ± 0.6	9.7 ± 1.7	11.2 ± 1.5
GIP								
fasting (pmol/l)	7.2 ± 1.7	5.6 ± 1.0	4.5 ± 1.0	8.5 ± 1.5*	6.7 ± 1.5	5.5 ± 1.1	4.6 ± 1.7	7.5 ± 1.2*
max (pmol/l)	50 ± 7.4	44 ± 6.4	15 ± 2.1**	22 ± 2.6**	36 ± 3.7	28 ± 3.5	18 ± 3.1**	14 ± 2.4**

Data are mean ± SEM. The mean fasting concentration was also considered when identifying the maximum (max) and minimum (min) value. *adjusted $p<0.05$ **adjusted $p<0.01$ vs. suc (within-group comparison using Wilcoxon signed rank test followed by Bonferroni adjustment for multiple comparisons). §adjusted $p<0.05$ §§adjusted $p<0.01$ vs. RYGB (between-group comparison of corresponding test days using Mann-Whitney test followed by Bonferroni adjustment for multiple comparisons). glu=glucose, fru=fructose, suc=sucrose, iso=isomaltulose, aca=acarbose, GLP-1=Glucagon-like Peptide-1, PYY=Peptide YY, GIP=Glucose-dependent Insulinotropic Polypeptide, AUC=area-under-the-curve, tAUC=total AUC, piAUC=positive incremental AUC (see figure 3 for piAUC of GLP-1, PYY and GIP).