Chapter 2

Calorie restriction is a major determinant of the short-term metabolic effects of gastric bypass surgery in obese type 2 diabetic patients

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ABSTRACT

Objective

Roux-en-y-gastric bypass (RYGB) and restrictive weight loss interventions, such as Gastric banding (GB) and very-low-calorie diets (VLCD) directly impact glucose metabolism, possibly by calorie restriction and/or altered secretion of gut hormones. We aimed to establish the direct endocrine and metabolic effects of RYGB compared to restrictive interventions in obese glucose-tolerant (NGT) subjects and subjects with type 2 diabetes (T2DM).

Methods

Four groups of obese females received a mixed meal at baseline and 3 weeks after intervention; NGT-GB (n=11), NGT-RYGB (n=16), T2DM-RYGB (n=15) and T2DM-VLCD (n=12). Normal weight controls (n=12) were studied once.

Results

At baseline, all obese subjects were hyperinsulinemic. T2DM was associated with hyperglycemia and decreased GLP-1 levels. RYGB and VLCD reduced glucose levels to a similar extent in T2DM, insulin levels decreased only after VLCD. Comparison of restrictive intervention versus RYGB, showed a more pronounced decrease in glucose and insulin AUC after restriction. In NGT and T2DM subjects, RYGB increased GLP-1 and PYY levels and decreased Ghrelin levels, whereas VLCD and GB only increased GIP levels.

Conclusions

These data indicate that deterioration of glucose metabolism in T2DM is associated with a decline of GLP-1 levels. Calorie restriction facilitates glucose metabolism and blunts hyperinsulinemia in obese (diabetic) humans. Additional duodenal exclusion through RYGB induces gut hormone release and hyperinsulinemia, but does not improve postprandial glucose levels any further. Our data thus strongly suggest that calorie restriction underlies the short-term metabolic benefits of RYGB in obese T2DM patients.

INTRODUCTION

Even though the pathogenesis of obesity and type 2 diabetes is incompletely understood, current scientific knowledge suggests that metabolic, neuroendocrine and inflammatory adaptations to overnutrition and inactivity are involved (1;2). Calorie restriction effectively improves glucose metabolism on the short term (3). Roux-en-Y gastric bypass surgery (RYGB) has also been shown to improve glucose metabolism within weeks, before significant weight loss has occurred (4;5). This suggests that gastrointestinal rearrangements and/or concomitant calorie restriction per se exert direct beneficial effects on metabolism (6-9).

Various reports have suggested that gut hormones, i.e. Glucagon-Like-Polypeptide-1 (GLP-1), Glucose-Dependent-Insulinotrophic-Peptide (GIP), peptide YY (PYY) and Ghrelin, are critical mediators of the benefits of bariatric surgery. GLP-1 and PYY, secreted by L-cells primarily located in de distal ileum, inhibit food intake and enhance glucose-dependent insulin secretion (10-12). Exaggerated postprandial GLP-1 and PYY secretion, driven by expedited nutrient delivery to L-cells, is hypothesized to confer the metabolic effects after RYGB (5). GIP, produced by K-cells in the duodenum (12), facilitates glucose-induced insulin release and promotes glucose uptake and fat storage in adipocytes. RYGB was reported to either increase (13) or decrease (14) GIP. Ghrelin is an orexigenic hormone, which is released by endocrine cells in the stomach in the absence of food and is suppressed by food intake (15). Although much debated, some evidence suggests that Ghrelin levels remain suppressed after RYGB, which possibly adds to the anorexigenic effect of this procedure (16). Finally, fatty acid entry into the proximal gut appears to play an important role in the control of endogenous glucose production via neural pathways in healthy (non-diabetic) rats (17).

In view of these multiple effects of hormones released by distinct gut segments in response to the presence or absence of food, it is conceivable that gastrointestinal rearrangements modifying food intake and/or the processing of nutrients do indeed have significant effects on postprandial hormone release and metabolism. However, RYGB also limits calorie intake to a substantial extent, and calorie restriction per se beneficially impacts postprandial metabolism. The relative contributions of calorie restriction versus the endocrine corollaries of bypass surgery to the metabolic benefits of RYGB remain elusive.

To clarify this issue, we conducted a clinical trial comparing the postprandial glucose, insulin and guthormone response to GB/calorie restriction or RYGB in obese

individuals with normal glucose tolerance and equally obese subjects with T2DM (trial no NCT01167959). We hypothesized, that gastric bypass surgery would increase postprandial gut hormone release to elevate insulin levels and lower glucose levels in response to a meal to a greater extent than calorie restriction per se. To identify effects that are independent of fat loss, we examined the endocrine and metabolic response to a mixed meal three weeks after intervention, when body weight loss was insignificant.

RESEARCH DESIGN AND METHODS

Subjects

Subjects were recruited from the waiting lists of several Dutch bariatric surgery centers, after referral for a weight loss program by their GP or internist. Screening by a multidisciplinary team of the Nederlandse Obesitaskliniek (Dutch Obesity Clinic) to establish if they fulfilled the international criteria for bariatric surgery as described by Fried *et al.* (18). Subjects eligible for dietary treatment were recruited after referral by their GP or internist. They fulfilled the same criteria as surgical patients but did not wish to undergo surgery yet. Exclusion criteria were smoking, age>65 years and any chronic disease other than diabetes, including psychiatric illness.

The subjects had either normal fasting glucose (NGT) or T2DM according to WHO standards. All diabetic subjects were treated with oral medication only (metformin, sulfonylurea derivatives). Subjects were excluded if weight loss medications had been used and/or or weight had not been stable within 90 days prior to enrollment of the study. Participants were allowed to use cholesterol lowering statins and antihypertensive medication.

Control subjects were recruited via an advertisement. They were all healthy females, age matched to the obese subjects, with a BMI in between 20-25 kg/m^2 and a normal plasma glucose concentration in fasting condition.

Ethics

The protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects provided written informed consent before participation.

Study design

Initially, we intended to include obese NGT and subjects with T2DM, who would have GB or RYGB to systematically compare the physiological effects of these interventions.

However, since RYGB was reported to have superior metabolic effects in subjects with T2DM, surgeons were reluctant to treat T2DM subjects with GB (4). Instead, we chose to include a group of with T2DM subjects who fulfilled the criteria for bariatric intervention, and treated them with a very-low-calorie diet (VLCD). As the effects of gastric banding presumably result primarily from calorie restriction, we reasoned that a VLCD might mimic the effects of GB.

Subjects were studied (after an overnight (10h) fast) within a month before surgery and again between 2 and 3 weeks after surgery. All oral glucose-lowering agents were discontinued 48 hours. After fasting blood samples had been taken, subjects were given 266 milliliters of a standardized fluid meal (Nutridrink®, 400 kcal; 49 energy % carbohydrate (48,9 g), 35% lipids (15,4 g), 16% protein (15,9 g)). Blood samples were drawn at the start of drinking (t=0) and 5, 10, 20, 30, 60, 90, 120, 150,180 minutes postprandial. Blood was collected in a SST® Gel and Clot Activator tube (Becton and Dickinson) and a vacutainer on EDTA. Serum Ghrelin, PYY 3-36, GIP and GLP-1 were collected in EDTA tubes with added Aprotinin (500 kallikrein inhibitory units/milliliter blood) or Dipeptidyl-Peptidase-IV (DPP-IV) inhibitor (10 microliter/milliliter blood; Linco, St. Charles, MO). EDTA tubes were immediately put on ice. All blood samples were centrifuged promptly (2000 g at 4 °C, for 10 minutes) and subsequently frozen (-80 °C) until assay.

Surgery

During RYGB, a 25 milliliters gastric pouch was created and connected to a 100cm Roux-en-Y limb. Gastric banding entailed placement of a standard silicone LapBand® (Inamed, Allergan, Santa Barbara, CA) around the stomach to create a 15 milliliters pouch. Patients were prescribed a clear liquid diet for 4-5 days (estimated 400 kcal/a day) after surgery, and were prescribed a staged meal plan afterwards. Retrospective assessment of their postoperative intake showed a mean intake of 700 kcal/day the second and third week post surgery.

Very low calorie diet

Commercially available Prodimed® (Prodimed Benelux BV, Valkenswaard, The Netherlands) is a high-protein-low-calorie meal replacement plan (VLCD), consisting of sachets (~90 kcal each, of which ~18 g protein, ~2.5-5 g carbohydrates, 0,5-2 g fat) soluble powder for preparation of meals. Subjects were allowed 4-5 sachets a day, and an additional choice of selected vegetables, resulting in an average calorie intake of 600 kcal/day.

Use of medication

At the day of operation or start of the VLCD, all blood glucose lowering agents were discontinued to avoid hypoglycaemia. Only Metformin treatment was reinstalled if fasting blood glucose levels remained above 7 mmol/L after intervention.

Assays

Serum glucose was measured using a Modular P800 chemistry analyzer (Roche Diagnostics, Mannheim, Germany; coefficient of variation (CV) 1.7%) . Insulin was measured with an immunometric assay on an automated Immulite 2500 (Siemens, Breda, The Netherlands; CV 6-7.5%). HbA1c was measured in whole blood samples using a High Performance Liquid Chromatography Integra 800 analyzer (Roche Diagnostics Mannheim, Germany; CV<2,5%). Total GIP was measured by enzyme-linked immuno-sorbent assay (ELISA) (EZHGIP-54K, Millipore, Billerica, USA; respectively inter-assay precision of 1.8–6.1% and intra-assay precision of 3.0–8.8%). Active GLP-1 was measured by ELISA (EGLP-35 K, Millipore, Billerica, USA; resp. 8 ± 4.8 % and 8.4 ± 1.1%). Human PYY was measured by radioimmunoassay (RIA) (PYY-67HK, Millipore, Billerica USA; resp. 6,4–11% and 7-15%). Total human Ghrelin was measured by RIA (GHRT-89 HK, Millipore, Billerica, USA; resp. 14.7–17.8% and 3.3–10.0%).

Assessment of insulin resistance

Insulin resistance was calculated with the Homeostatic model assessment (HOMA-IR): *Fasting insulin (mU/milliliters)* fasting glucose (mmol/l) / 22,5.* Matsuda index, a measure of insulin insensitivity obtained from oral glucose or meal testing, was calculated with the official calculator application from http://mmatsuda.diabetes-smc.jp/english.html.

Statistical analysis

Data were analyzed using SPSS 17.0. Data are presented as means ± standard error of the mean. Baseline differences among NGT, T2DM subjects and controls were calculated using Univariate ANOVA. The effects of the different interventions were compared with a mixed-effects model, with the patient groups and diabetes as fixed effects and the subject specific deviances modelled with random intercepts. The Bonferroniposthoc test was used to correct for multiple testing. A p-value <0,05 was considered statistically significant. Graphs were developed in Prism Graph Pad 5.

Table 1 - Baseline characteristics of study groups.

	1.NGT-GB (n=11)	2.NGT-RYGB (n=16)	3.T2DM-RYGB (n=15)	4.T2DM-VLCD (n=12)	5.Controls (n=12)	
Age (yrs)	46.3 ±1.9	48.6 ± 1.6	51.3 ± 1.9	50.8 ± 2.1	49.2 ± 1.8	
Weight (kg)	118.6 ± 3.9	128.2 ± 2.3*	121.3 ± 4.1	112.0 ± 5.1*	64.3 ± 2.1**	*p<0.05 (2 vs.4)
BMI (kg/m²)	43.1 ± 0.9	44.2 ± 0.8	43.5 ± 1.1	40.2 ± 1.9	21.7 ± 0.5**	
WHR (cm)	0.87 ± 0.0	0.87 ± 0.0	0.91 ± 0.0	0.93 ± 0.0	0.79 ± 0.0**	
T2DM duration (yrs)	-	-	4.2 ± 0.7	3.4 ± 0.7	-	
HbA1c (mmol/mol)	35.3 ± 1.6	36.8 ± 2.3	53.2 ± 3.6	45.1 ± 2.1	31.9 ± 0.7	*p<0.05 (1.2.5 vs.3.4) *p<0.05 (3 vs.4)
Fasting glucose (mmol)	5.0 ± 0.2	5.0 ± 0.1	8.9 ± 0.6	8.4 ± 0.8	4.9 ± 0.1	*p<0.05 (1.2.5 vs.3.4)
Fasting insulin (mU)	11.5 ± 2.3	9.8 ± 1.9	10.8 ± 1.9	13.5 ± 2.4	1.6 ± 0.0**	
HOMA-ir	2.6 ± 1.6	2.3 ± 0.5	4.4 ± 0.8	4.9 ± 0.8	0.3 ± 0.0*	

Values are means ± SEM. P-values indicate significant differences between groups at baseline. A Bonferoni correction was used to allow for multiple comparisons between groups at baseline. **p<0.001 between obese patients and lean controls, if not otherwise indicated; *p<0.05 between obese patient groups at baseline,if not otherwise indicated (groups indicated between brackets).

Table 2 – Effects of intervention on weight. fasting and meal stimulated (area under the response curve) plasma levels of glucose, insulin and of gut peptides.

	1. NGT-GB (n=11) Baseline - After Intervention		2. NGT-RYGB (n=16) Baseline-After Intervention	
Weight (kg)	118.6 ± 3.9	113.1 ± 4.2*	128.2 ± 2.3*	119.4 ± 2.6*
BMI (kg/m²)	43.1 ± 0.9	40.5 ± 0.9*	44.2 ± 0.8	40.9 ± 0.8*
HOMA-ir	2.6 ± 1.6	1.6 ± 0.5	2.3 ± 0.5	1.9 ± 0.4
Matsuda index	7.0 ± 2.0	13.8 ± 3.2*	*9.3 ± 2.2	8.7 ± 1.8
Glucose(f) (mmol)	5.0 ± 0.2	4.9 ± 0.2	5.0 ± 0.1	4.8 ± 0.2
AUC glucose (mmol/l/3h)	038 ± 62	956 ± 56	1036 ± 35.8	1039 ± 46
Peak Glucose (mmol/l)	6.9 ± 0.4	6.7 ± 0.4	6.9 ± 0.3	8.2 ± 0.4*#
Time to peak Glucose (min)	68.2 ± 10.0	70.9 ± 8.9	89.4 ± 8.3	38.1 ± 6.5*#
Insulin(f) (mU)	11.5 ± 2.3	6.9 ± 1.9*	9.8 ± 1.9	8.7 ± 2.3
Peak Insulin (mU/l)	90.9 ± 21.4	64.2 ± 11.8	58.7 ± 6.4	141.6 ± 28.3*#
Time to peak Insulin (min)	73.6 ± 8.4	84.1 ± 10.2	91.9 ± 6.9	33.8 ± 9.1*#
AUC Insulin (mU/l/3h)	9007 ± 2403	5913 ± 923	6200 ± 842	7926 ± 1567
GLP-1(f) (pM)	3.8 ± 1.0	2.5 ± 0.7*	3.0 ± 0.6	2.8 ± 0.6
Peak GLP-1 (pmol/l)	4.9 ± 0.9	5.4 ± 0.9	5.4 ± 0.8	49.1 ± 4.9*#
Time to peak GLP-1 (min)	76.3 ± 15.4	50.0 ± 7.4	65.6 ± 12.8	28.1 ± 6.1*
AUC GLP-1 (pmol/l/3h)	713 ± 170	655 ±113	647 ±105	3161 ± 483*#
GIP(f) (pg)	28.6 ± 9.1	32.7± 7.2	44.6 ± 7.5	27.7 ± 5.7
Peak GIP (pmol/l)	297 ± 27	384 ± 37*	234 ± 27	386 ± 31*
Time to peak GIP (min)	57.3 ± 7.5	87.2 ± 7.5*	65.6 ± 6.2	30.0 ± 6.3*
AUC GIP (pg/l/3h)	30727±3245	48495±15836*	24291±2690	30950±2949*#
PYY(f) (pM)	88.0 ± 10.2	74.9 ± 9.5	70.8 ± 8.5	66.8 ± 8.9
Peak PYY (pmol/l)	104.5 ±11.6	123.8 ±17.2	98.9 ± 9.6	185.9±14.3*#
Time to peak PYY (min)	68.1 ± 17.0	81.8 ± 12.1	77.5 ± 14.2	76.9 ± 10.0
AUC PYY (pmol/l/3h)	15619±1535	17120±2129	13590±1272	25983±1765*#
Ghrelin(f) (pg)	538.5 ± 70.6	521.1 ± 65.8	734.4 ± 72.0	511.1 ± 67.0*
AUC Ghrelin (pg/l/3h)	80678±8567	86275±8694	106821±9729	72613±8174*

Values are means±SEM. *p<0,05; within groups before and after intervention. **p<0,001 between patient groups and controls at baseline. # p<0,05 significant different effect of different interventions between either T2DM groups or NGT groups.

3. T2DM-RYGB (n=15) Baseline - After Intervention		4. T2DM-VLCD (n=12) Baseline - After Intervention		5. Controls (n=12)
121.3 ± 4.1	112.5 ± 3.9*	112.0 ± 5.1*	105.3 ± 4.8*	64.3 ± 2.1**
43.5 ± 1.1	40.4 ± 1.1*	40.2 ± 1.8	37.7 ±1.7*	21.7 ± 0.5
4.4 ± 0.8	3.3 ± 0.5#	4.9 ± 0.8	1.1 ± 0.2*#	0.3 ± 0.0**
5.3 ± 1.1	5.5 ± 1.3	*3.9 ± 0.6	11.8 ± 2.0*#	26.5 ± 3.8**
8.9 ± 0.6	6 ± 0.4*	8.4 ± 0.8	5.2 ± 0.3*	4.7 ± 0.1
1899 ± 147	1479 ± 99*	1774 ± 152	1346 ± 76*	952 ± 32
12.6 ± 0.9	10.9 ± 0.6*	11.8 ± 0.9	9.3 ± 0.6*	6.5 ± 0.2
84.0 ± 8.6	36.0 ± 5.8 *	95.0 ± 9.6	87.5 ± 9.3 #	60.8 ± 9.6**
10.8 ± 1.9	10.9 ± 1.6#	13.5 ± 2.4	4.9 ± 0.8*#	1.6 ± 0.0**
52.3 ± 6.9	86.3 ± 18.5 *	48.8 ± 6.8	30.9 ± 3.1*#	29.2 ± 4.5
102.0 ± 7.2	42.0 ± 8.8 *	00.0 ± 8.0	120.0 ± 7.8 #	76.7 ± 8.0
5663 ± 749	6781 ± 1255	6018 ± 827	3894 ± 488*	2278 ± 277**
1.9 ± 0.4	1.5 ± 0.2	1.6 ± 0.4	1.4 ± 0.4	1.7 ± 0.2
4.4 ± 0.8	44.5 ± 7.9*	3.8 ± 0.9	4.0 ± 0.7#	5.3 ± 0.9
66.0 ± 13.2	25.3 ± 6.3*	47.5 ± 14.7	57.5 ± 7.0	54.2 ± 14.7
526 ± 96	2383 ± 343 *	396 ± 72	395 ± 87#	560 ± 76
45.4 ± 7.8	31.5 ± 5.9	45.7 ± 8.7	41.6 ± 6.7	39.3 ± 6.6
288 ± 30	441 ± 39*	261 ± 21	337 ± 18*	274 ± 24
60.0 ± 6.4	34.0 ± 6.5*	55.8 ± 7.2	70 ± 7.2	58.3 ± 7.2
31019±2778	34611±2841	30964±3106	41636±2404*	32799±3106
70.7 ± 8.7	56.7 ± 5.0*	114.8 ± 9.8	110.1 ± 9.7	87.2 ± 10.7
77.9 ± 10.0	165.8±14.7*	126.6±11.1	132.3 ±16.5#	126.5 ± 16.5
110.7 ± 14.6	68.0 ± 10.3*	73.3 ± 16.4	115.0 ± 11.5#	63.3 ± 11.5
12255±1314	22548±1823*	20227±1469	20394±2038#	17240±2038
563.5 ± 72.2	452.5 ± 65.6*	670.1 ± 83.7	613.7 ± 79.7	1147.5 ± 97.5**
84501±9698	69236±8712*	99204±12896	87044±9916*	159878±12105**

RESULTS

Subject characteristics

All obese subjects and healthy controls were Caucasian females, with a mean age of 49,4 ± 0,6 yrs (table 1). We included 32 subjects with T2DMand 30 NGT obese individuals. Mean BMI was comparable among the obese groups at baseline. Eight subjects dropped out during the course of the study because they were not able to comply with the VLCD group (n=2), because of practical issues (n=3); and because of mild postoperative complications (n=3).

Weight loss and intake

Weight loss was similar in NGT and T2DM groups at 3 weeks after either intervention (table 2). Conceivably, three weeks after treatment all subjects were still markedly obese (table 2).

Medication use

Metformin treatment was reinstalled in T2DM subjects if fasting blood glucose levels remained above 7 mmol/L after intervention (27% of subjects after RYGB vs.17 % of subjects after VLCD, p=ns).

Baseline plasma values

Fasting plasma levels of HbA1c, glucose and insulin are shown in table 1. Glucose AUC was higher in T2DM subjects, coinciding with a trend towards decreased insulin and GLP1 AUC as compared to obese NGT subjects (table 2, figure 1). AUC insulin was higher and Ghrelin levels were approximately 30% lower in all obese subjects compared to lean controls.

Effects of intervention

RYGB vs. GB in NGT subjects

Neither GB nor RYGB did affect fasting or postprandial glucose levels. However, there was a significant increase in peak glucose after RYGB (table 2, figure 1). GB decreased fasting ($p<0,05$) and postprandial insulin levels by 30%, whereas postprandial levels increased after RYGB. Postprandial GLP-1 and PYY levels were not affected by GB, but RYGB induced a leftward shift of the response curve, a decreased time to peak, and a five- and twofold increase of total AUC of both hormones respectively (table 2, figure 2,3). The increase in AUC-GIP after GB was more pronounced as compared to after RYGB ($p<0,05$). Ghrelin was more suppressed by the meal after RYGB, but not after GB (supplemental figure S1).

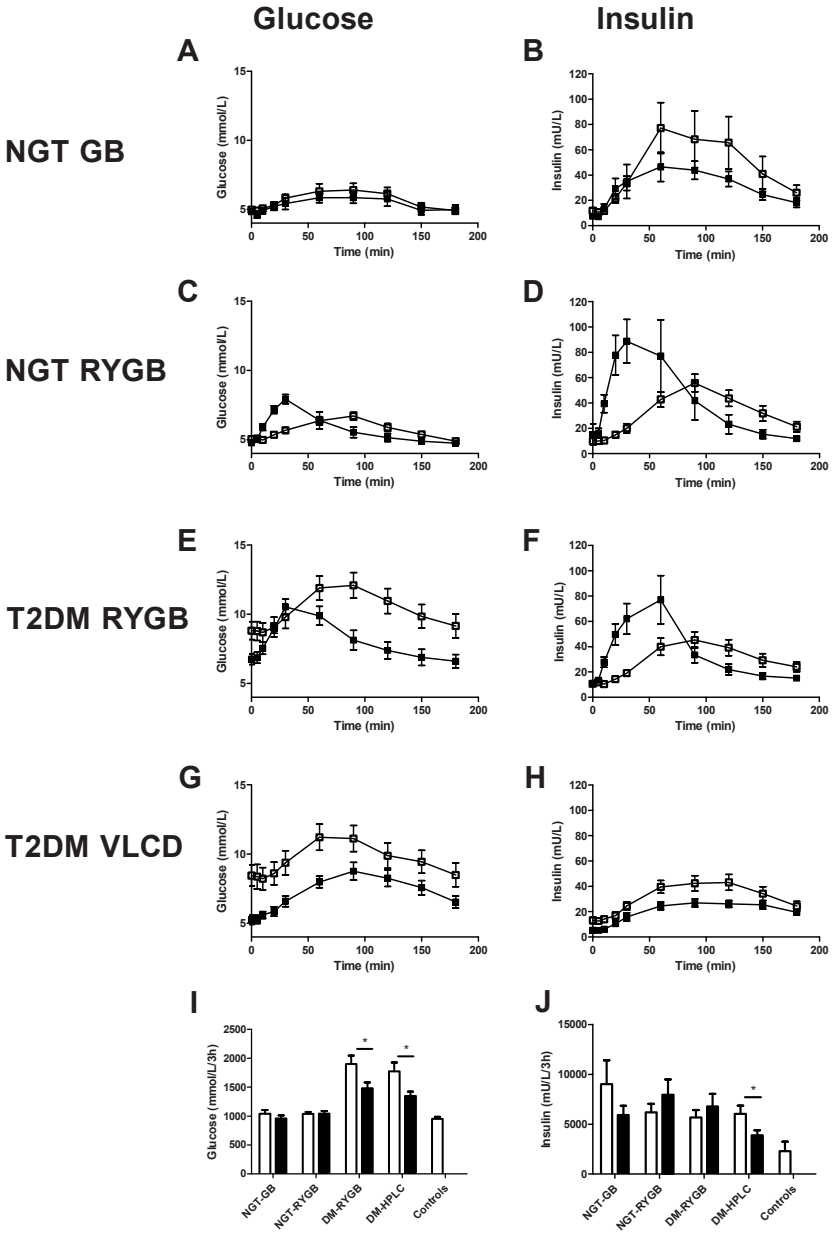

Figure 1 - Glucose and insulin concentrations during mixed meal tolerance test before and intervention.

Glucose and insulin concentrations during mixed meal tolerance test before (open squares) and after intervention (closed squares) in NGT-GB group(A,B), NGT-RYGB group(C,D), T2DM-RYGB group(E,F), T2DM-VLCD group(G,H) and AUC glucose and insulin before (white bars) and after (black bars) (I,J) intervention. Values are means ± SEM.

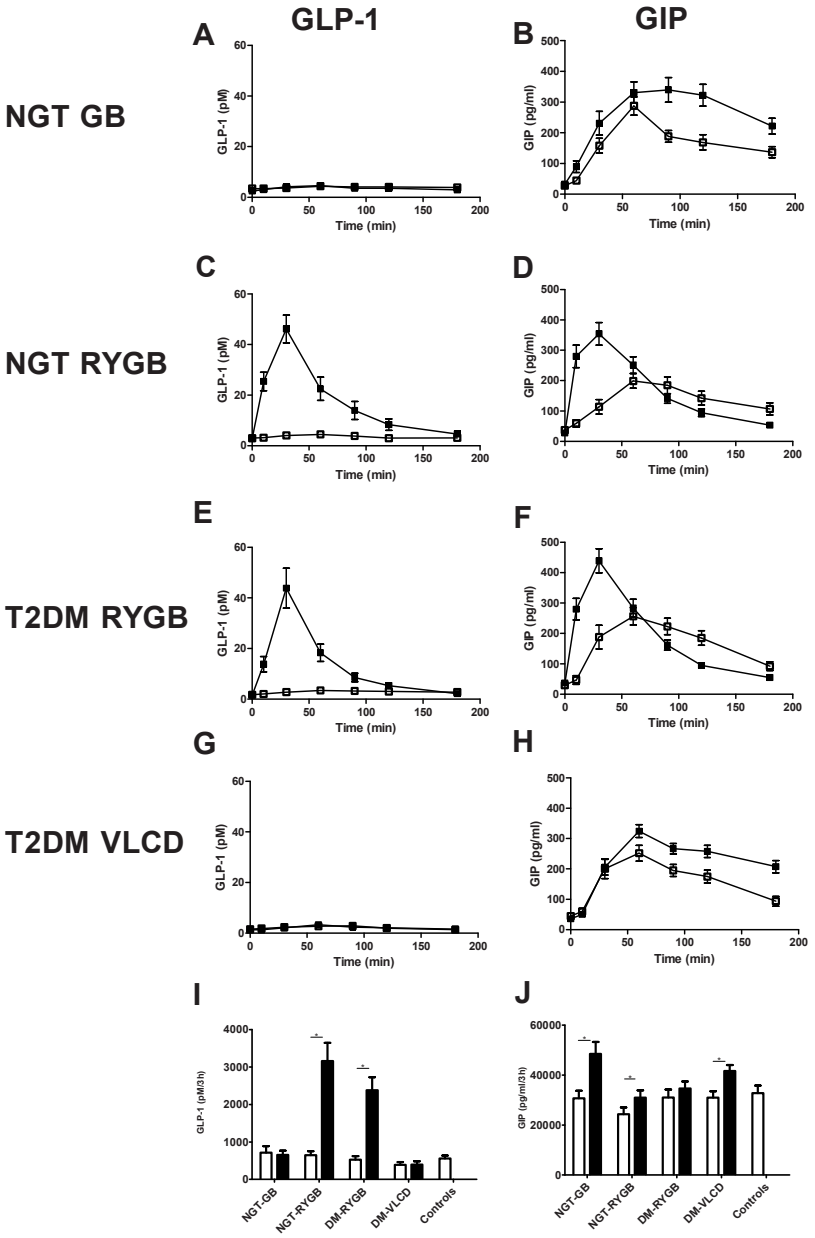

Figure 2 - GLP-1 and GIP concentrations during mixed meal tolerance test before and after intervention.

GLP-1 and GIP concentrations during mixed meal tolerance test before (open squares) and after intervention (closed squares) in NGT-GB group(A,B), NGT-RYGB group(C,D), DM-RYGB group(E,F), DM-VLCD group(G,H) and AUC GLP-1 and GIP before (white bars) and after (black bars) (I,J) intervention. Values are means ± SEM.

RYGB vs VLCD in T2DM subjects

RYGB and VLCD induced a comparable decline in fasting and postprandial glucose levels, but a significant shortening of time-to-peak glucose was observed after RYGB and not after the VLCD (table 2). Fasting insulin levels decreased only after VLCD. Homa-ir significantly decreased after VLCD, but not after RYGB.

AUC insulin levelsincreased after RYGB and decreased by 30% after VLCD. Concomitantly, only RYGB increased postprandial AUC and peak level and shortened time to peak of both GLP-1 and PYY. The Matsuda index increased significantly after VLCD, whereas there was no effect after RYGB.

DISCUSSION

This is the first study to extensively compare the effects of RYGB with those of restrictive weight loss strategies on postprandial glucose and gut peptide metabolism inobese humans with and without T2DM in parallel. We aimed to evaluate the direct effects of intervention, since RYGB appears to have profound metabolic benefits apart from its impact on body adiposity.Therefore, the subjects were examined right before and approximately three weeks after surgery or initiation of calorie restriction, when relatively little weight was lost. On both occasions, they consumed the same mixed meal to evoke a physiological response of metabolites and peptides.

In aggregate, our data indicate that obesity is associated with hyperinsulinemia, a reduction of plasma ghrelin levels, and an increase in plasma GLP-1 levels, while other gastrointestinal peptide concentrations appear normal. Deterioration of glucose metabolism in T2DM is associated with a decline in circulating GLP-1. In NGT subjects, gastric banding reduces postprandial insulin levels without profound changes in gut peptide secretion. RYGB does not affect glucose metabolism any further in these subjects, despite enhancement of the insulin response and distinct changes in gut hormone levels. In T2DM patients, caloric restriction alone and RYGB are associated with equivalent reductions in fasting and postprandial hyperglycaemia. Apparently, rearrangement of gastrointestinal architecture through RYGB profoundly elevates postprandial GLP-1, PYY and GIP and blunts ghrelin concentrations, but does not improve glucose metabolism any more than calorie restriction per se.

The metabolic responses to GB (in NGT) and VLCD (in DM2) we observed were remarkably similar. Indeed, both GB and VLCD (tended to) reduce fasting and

postprandial glucose and insulin levels as soon as 3 weeks after intervention, when only little weight was lost. This suggests that calorie restriction per se determines the beneficial effects of GB and VLCD. It was shown before that only two days of very low calorie feeding reduces endogenous glucose production in obese diabetic patients (3). Recent evidence suggests that reduction of nutrient entry into the proximal gut may be mechanistically involved in this process. Upper intestinal lipids suppress glucose production in rats, and this mechanism is desensitized by high fat feeding (17). Conversely, calorie restriction may restore proper functioning of this mechanism to reduce endogenous glucose production in response to a meal.

The effects of RYGB on glucose metabolism have been reported to occur within days after surgery (4;19;20). In addition to gastric volume reduction, which limits calorie intake, RYGB entails rearrangement of gastrointestinal architecture, which involves profound changes in nutrient processing and postprandial gut hormone release. Specifically, the expeditious delivery of nutrient-rich chyme to jejunal and ileal L-cells is supposed to exaggerate postprandial GLP-1 and PYY secretion, thereby enhancing insulin secretion and satiety, ultimately ameliorating glucose intolerance. We confirm these effects and observed a marked increase in postprandial insulin, however, without any significant change of (postprandial) glucose metabolism in obese NGT individuals. In DM2 patients, postprandial glucose levels were reduced in the face of similar (if not slightly elevated) insulin levels after RYGB. These data may fit with the notion that RYGB impacts on postprandial glucose metabolism through gut peptide mediated stimulation of insulin release. However, careful comparison of the effects of GB/calorie restriction and RYGB on postprandial metabolism suggests otherwise.

First of all, calorie restriction alone was as effective in lowering postprandial glucose levels in diabetic patients as RYGB. As Ilsbell *et al.* described (21) improvement of glucose metabolism is achieved by administration of the "post-RYGB" diet only. Moreover, in our subjects the metabolic improvement in response to calorie restriction alone was accomplished in the face of significantly reduced insulin levels, whereas postprandial insulin concentrations tended to increase after RYGB. Furthermore, the Matsuda index, which reflects insulin sensitivity (albeit not as accurate as a euglycemic clamp) increased after restriction, but not after RYGB. Thus, it appears that *more* insulin is required to maintain euglycemia after RYGB, than in subjects who simply restrict their calorie intake. There are at least 2 potential explanations for this observation. The bypass procedure prevents nutrient entry into the duodenum, where, as alluded to before, nutrient sensing systems activate a gut-brain-liver axis to inhibit postprandial

glucose production (17). We speculate that bypassing this system by a RYGB procedure may hamper neural suppression of glucose output, triggering insulin release (in concert with excess incretins) to maintain normoglycemia. Alternatively, the surgical procedure per se may hamper insulin action (22), but simultaneously promote gut peptide release in response to a meal, thereby stimulating insulin release, which offsets any deleterious impact on insulin sensitivity, ultimately lowering glucose concentrations to an extent similar to that brought about by calorie restriction per se.

To further compare the metabolic effects of RYGB versus calorie restriction per se, we analyzed the differences between all subjects receiving RYGB vs those receiving GB/VLCD (table 3). It should be emphasized that although both groups comprised equal numbers of NGT and T2DM subjects, the comparison provides just a rough impression, because of the metabolic mix of subjects in each group and the implicit premise that GB primarily acts through its restrictive quality. However, this comparison adds to the notion that calorie restriction per se improves insulin sensitivity (HOMA-ir and matsuda index) and moreover, evokes a more pronounced decrease in postprandial glucose levels.

Given all this, we believe that our data suggest that the immediate beneficial effects of RYGB on postprandial glucose metabolism are primarily due to calorie restriction brought about by gastric volume reduction. We showed that elevated meal-induced gut hormone release in response to RYGB, which stimulates insulin secretion, does not lower postprandial glucose levels any more than calorie restriction per se. We suggest that RYGB-induced incretin-stimulated insulin release is in fact required to offset detrimental effects of the bypass procedure on insulin sensitivity and/or neural control of endogenous glucose production. The long term metabolic benefits of RYGB over GB are probably due to its profound effects on gut hormones, promoting satiety and weight loss (23). This notion is supported by the fact that resolution of T2DM in the long run after bariatric surgery is, among other factors, dependent on the amount of weight lost (24;25).

Dietary composition in response to intervention was most likely different among groups. However, an estimation of calorie intake was broadly comparable, and moreover, an equivalent decrease in BMI in subjects after surgery or VLCD suggests that calorie intake was roughly similar. Also, it seems important to emphasize that our main outcome parameter, postprandial glucose levels, was measured after the exact same test meal in all four study groups.

In conclusion, our data reveal clearly distinct sub-acute effects of calorie restrictive interventions *versus* Roux-en-Y gastric bypass surgery on meal-induced gut hormone and insulin concentrations in obese humans, whilst effects on postprandial glucose levels are similar among procedures. We suggest that restriction of calories is of paramount importance for the early metabolic benefits of RYGB, and that additional bypass of the proximal gut might even impair postprandial glucose metabolism (through disruption of neural suppression of glucose production and/or through induction of insulin resistance), which is offset by incretin-induced insulin secretion.

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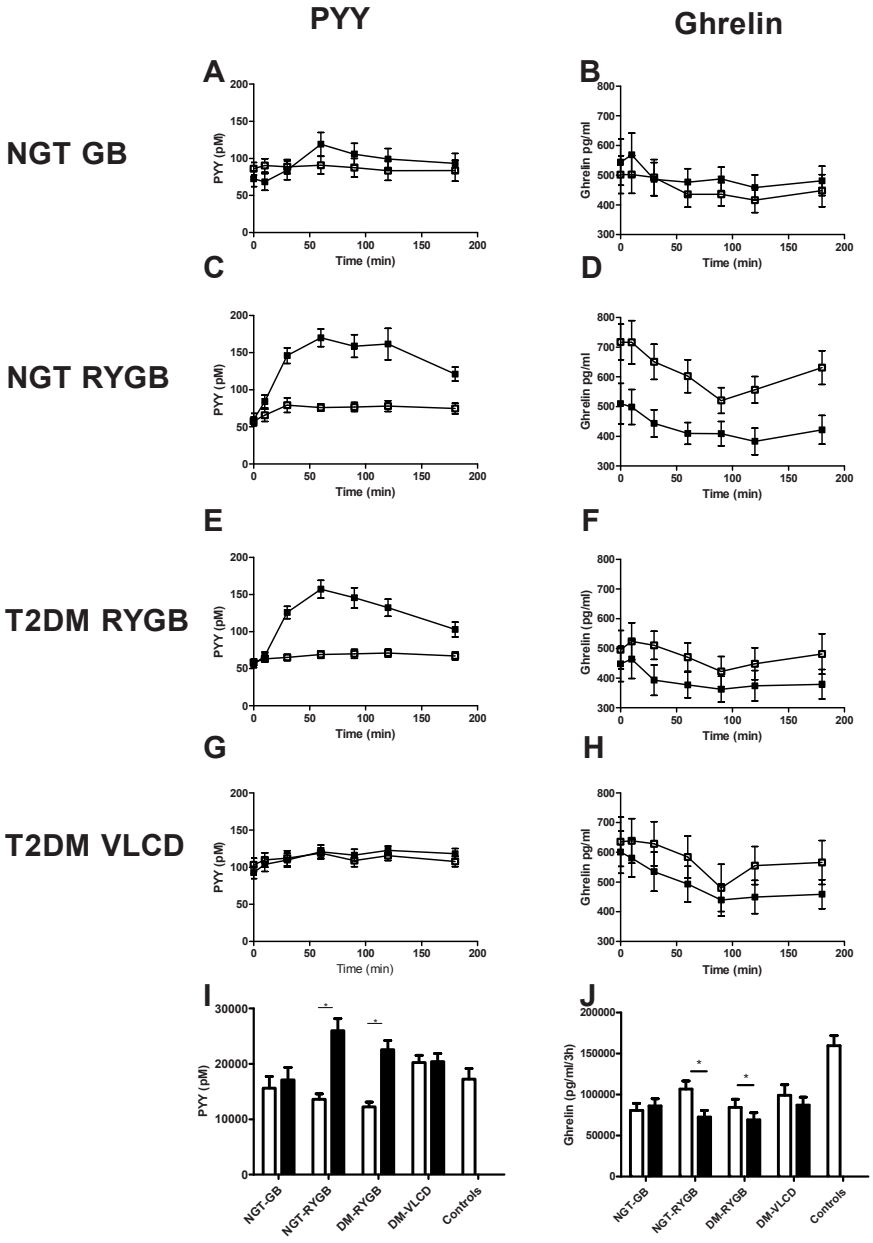

Supplemental Figure S1 - PYY and Ghrelin concentrations during mixed meal tolerance test before and after intervention.

PYY and Ghrelin concentrations during mixed meal tolerance test before (open squares) and after intervention (closed squares) in NGT-GB group(A,B), NGT-RYGB group(C,D), DM-RYGB group(E,F), DM-VLCD group(G,H) and AUC GLP-1 and GIP before (white bars) and after (black bars) (I,J) intervention. Values are means ± SEM.