Chapter 3

Roux-en-Y gastric bypass, but not calorie restriction, increases postprandial glucagon release in obese women with or without type 2 diabetes

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ABSTRACT

Objective

Glucagon may add to the pathogenesis of hyperglycemia in type 2 diabetes mellitus (T2DM). Glucagon secretion is, among other factors, determined by GIP and GLP-1, gut hormones which are suggested to improve glucose tolerance after roux-en-y-gastric bypass (RYGB). The aim of this study was to determine the postprandial glucagon response in obesity with normal glucose tolerance (NGT) and T2DM and the effects of RYGB as compared to calorie restriction, as induced by a very low calorie diet (VLCD) or gastric banding (GB).

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. Glucose, insulin, glucagon and gut peptide levels were measured until 180 minutes postprandially.

Results

At baseline, glucagon levels were comparable between obese groups, but significantly higher in obese as compared to lean subjects. T2DM was associated with postprandial hyperglycaemia and hyperglucagonemia. Calorie restriction and RYGB both decreased postprandial glucose levels in T2DM subjects. After RYGB, this was accompanied by hyperinsulinemia and an increase in GLP-1 and glucagon secretion.

Conclusions

These data indicate that the gastrointestinal rearrangements (but not calorie restriction) of RYGB are responsible for the concerted increase of postprandial insulin, GLP-1 and glucagon levels that mark this procedure.The parallel increase of postprandial glucagon and GLP-1 levels after this procedure may promote weight loss and metabolic benefits in the long term.

INTRODUCTION

The gut plays an important role in postprandial glucose metabolism. Nutrient sensing by the gut, pancreas and brain and the adaptive secretion of gut peptides regulating postprandial metabolism, maintain glucose levels in tight equilibrium (1;2). Glucagon is involved in the maintenance of normoglycemia in the post-absorptive state. The post-absorptive decline of circulating insulin and glucose levels unleashes glucagon secretion by pancreatic alpha-cells, thereby stimulating hepatic glucose production in order to maintain normoglycemia (3). Postprandial glucagon release is blunted by hyperglycemia, hyperinsulinemia and elevated levels of glucagon-like-peptide-1 (GLP-1), to compensate for the glucagonotropic effects of glucose dependent insulinotropic polypeptide (GIP) (4).

In type 2 diabetes (T2DM), plasma glucagon levels are inappropriately high in the context of ambient hyperglycemia and hyperinsulinemia (3), potentially deteriorating metabolic control (5). Furthermore, whereas normal suppression of glucagon is observed after intravenous glucose administration (bypassing the gastrointestinal tract), there is a lack of adequate glucagon suppression after isoglycemic orally administered glucose in T2DM (6). This suggests that gut derived factors, such as increased secretion of glucagonotropic GIP, reduced secretion of glucagonostatic GLP-1, or reduced alpha-cell inhibition by insulin are responsible for postprandial hyperglucagonemia in T2DM (7;8). Insufficient or delayed inhibition of glucagon release can contribute to postprandial hyperglycemia in T2DM (4;9;10). It has therefore been suggested that inadequate glucagon regulation is involved in the early pathogenesis of type 2 diabetes (11).

Roux-en-Y Gastric Bypass (RYGB) surgery has proven to be very effective in the achievement of long- term weight loss and glucose control in T2DM. Various reports have suggested that the gastro-intestinal rearrangements established by this procedure are critical mediators of these benefits by stimulating the secretion of insulinotropic gut peptides in response to food intake. (12) Whether or not glucagon secretion is also affected by RYGB is less well documented. Only a few studies examined the postprandial glucagon response in parallel with profiles of gut hormones in obese subjects with T2DM (13). Moreover, given the putative importance of gut derived peptides modulating glucagon release in the effects of RYGB on metabolism, it is important to define the effects of this procedure on glucagon levels.

The aim of this study was to compare the postprandial glucagon response (and its regulatory gut peptides) in obese T2DM subjects as compared to equally obese

subjects with normal glucose tolerance and, moreover, to study the effects of RYGB as compared to calorie restriction on postprandial glucagon concentrations in these subjects.

METHODS

The research design and methods have been described in detail elsewhere (14). In short, we included obese females, with normal fasting glucose (NGT) or T2DM (treated with metformin or SU derivatives only), eligible for both dietary and surgical treatment.. Control subjects were lean, healthy females. The protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects provided written informed consent before participation.

Subjects were studied (after an overnight (10h) fast) within a month before surgery and between 2 and 3 weeks after surgery. Fasting and postprandial (266 milliliters Nutridrink®, 400 kcal; 49 energy % carbohydrate (48,9 g), 35% lipids (15,4 g), 16% protein (15,9 g)) blood samples were taken. Blood was collected in a SST® Gel and Clot Activator tube (Becton and Dickinson) and a vacutainer on EDTA with added Aprotinin or Dipeptidyl-Peptidase-IV (DPP-IV) inhibitor as appropriate (14).

Interventions

Standard operating procedures were followed for GB and RYGB and patients were prescribed a staged meal plan after surgery (14). VLCD subjects were prescribed commercially available Prodimed® (Prodimed Benelux BV, Valkenswaard, The Netherlands), a high-protein-low-calorie meal replacement plan(VLCD) (14).

Assays

Glucagon was measured by radioimmunoassay (RIA) (Glucagon GL-32K, Millipore, Billerica USA) with an inter-assay precision of 4.0% and an intra-assay precision of 7.3%. This assay is specific for pancreatic glucagon, and cross reactivity to oxyntomodulin is less than 0,1%. Serum glucose, insulin, HbA1c, GIP and GLP-1 were analyzed and HOMA-IR calculated as described (14).

Statistical analysis

Data were analyzed using SPSS 17.0. Data are presented as means ± SE. AUC was calculated as increase from fasting level. Differences among groups at baseline and the effects of the interventions were compared with a mixed-effects model, with the

patient groups and diabetes as fixed effects and the subject specific deviances modeled with random intercepts. A p-value <0,05 was considered statistically significant. The Bonferroniposthoc test was applied to correct for multiple testing. After correction (15 parameters and 9 contrasts) a p<0,00037 was considered significant. An uncorrected trend was defined as a p-value <0,05. Correlations in AUC parameters between groups after restriction versus groups after RYGB were corrected for multiple testing separately: after correction for 5 parameters (10 in symmetry) and 2 contrasts this resulted in a significance cut-off p<0,0025. Graphs were developed in Prism Graph Pad 5.

RESULTS

Subjects, weight loss and medication use

All subjects were Caucasian women with a mean age of 49.4 ± 0,6 yrs. Weight loss was similar in NGT and T2DM groups at 3 weeks after either intervention (table 1, ref (14)). According to protocol, Metformin treatment was reinstalled in T2DM subjects if fasting blood glucose levels remained above 7 mmol/L (27% of subjects after RYGB vs.17 % of subjects after VLCD, p=ns).

Differences in hormones and metabolites between lean, obese NGT and obese T2DM subjects

Fasting

Fasting glucagon levels were increased in obese subjects as compared to lean controls. Fasting levels of hormones and metabolites are shown in table 1.

Postprandial

AUC glucagon was enhanced in obese T2DM subjects as compared to obese NGT (p=3.21E-11) and lean controls (p=7.23E-15; table 1, figure 1).Glucose AUC was increased in T2DM subjects as compared to Lean (p=1.53E-5) and NGT (p=4.68E-5), whereas AUC insulin was increased in NGT obese subjects compared to lean (p=0,009; table 1).

Effects of interventions on hormones and metabolites

Fasting

Fasting glucagon levels were unaffected by the interventions (table 2). Fasting glucose levels decreased after RYGB and the VLCD in T2DM subjects (resp p=8.80E-8; p=6.68E-13) (14). Insulin level only decreased after the VLCD (p=1.66E-5).

Table 1 - Baseline characteristics of the study groups.

	NGT (27)	T2DM (27)	Controls (12)	NGT vs Lean	T2DM vs Lean	T2DM vs NGT
BMI (kg/m^2)	43.8 ± 0.6	42.0 ± 1.1	21.7 ± 0.5	**8.41E-24**	**5.62E-22**	0.119133
Waist (cm)	122.2 ± 1.7	123.2 ± 2.1	78.0 ± 2.7	**3.3E-21**	**1.08E-21**	0.702118
HbA1c (mmol/mol)	36.1 ± 1.5	49.6 ± 2.1	31.9 ± 0.7	0.156349	**3.71E-08**	**7.96E-08**
HOMA-IR	2.4 ± 0.4	4.6 ± 0.6	0.3 ± 0.0	0.004094*	**3.01E-08**	**0.000149**
FFA (mmol/L)	1.0 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	0.284697	0.016636*	0.086563
Fasting glucose (mmol/L)	5.0 ± 0.2	8.7 ± 0.3	4.7 ± 0.1	0.563268	**8.52E-13**	**4.51E-16**
Fasting insulin (mU/L)	10.5 ± 1.3	12.0 ± 1.5	1.6 ± 0.1	**0.000298**	**3.21E-05**	0.435538
Fasting glucagon	63.0 ± 4.5	62.5 ± 3.8	46.5 ± 3.4	**4.86-05**	**6.96E-05**	0.932932
AUC glucose (mmol/L/3h)	152 ± 18	300 ± 24	99.0 ± 36.9	0.229553	**1.53E-05**	**4.68E-05**
AUC insulin (mU/L/3h)	5470 ± 917	3739 ± 414	2081 ± 283	0.009358*	0.198205	0.087537
AUC glucagon	541 ± 142	1114 ± 208	349 ± 73	0.366404	**7.23E-15**	**3.21E-11**

Values are presented as means ± SEM. Differences between subject groups (NGT vs. T2DM) and lean controls at baseline were compared with Mixed model. The Bonferroni posthoc test was used to correct for multiple testing: after correction a p-*value <0.00037* (bold values) was considered statistically significant. A trend (not significant after correction) was defined as p<0,05 and marked by *. Abbreviations: BMI: body mass index; FFA: free fatty acids; NGT: normal glucose tolerant; DM: type 2 diabetes mellitus; AUC: area under the response curve (as calculated from fasting level).

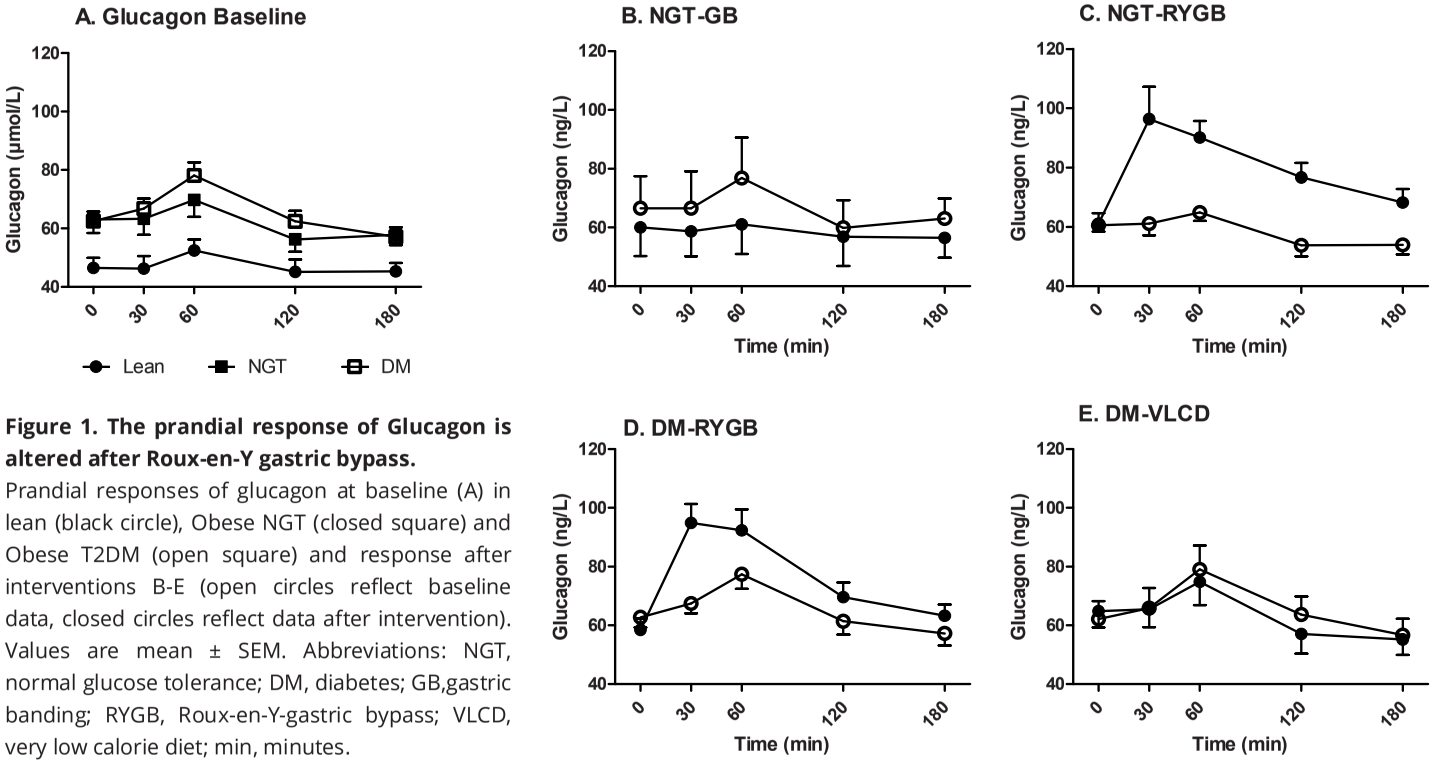

Figure 1. The prandial response of Glucagon is altered after Roux-en-Y gastric bypass.
Prandial responses of glucagon at baseline (A) in lean (black circle), Obese NGT (closed square) and Obese T2DM (open square) and response after interventions B-E (open circles reflect baseline data, closed circles reflect data after intervention). Values are mean ± SEM. Abbreviations: NGT, normal glucose tolerance; DM, diabetes; GB,gastric banding; RYGB, Roux-en-Y-gastric bypass; VLCD, very low calorie diet; min, minutes.

Table 2 – Effects of intervention on fasting and meal stimulated (area under the response curve) plasma levels of glacagon.

	NGT					T2DM				
	Baseline	**After GB (11)**	**p-value**	**After RYGB (16)**	**p-value**	**Baseline**	**After RYGB (15)**	**p-value**	**After VLCD (12)**	**p-value**
BMI (kg/m^2)	43.8 ± 0.6	40.5 ± 0.9	**9.52E-10**	40.9 ± 0.8	**2.9E-15**	42.0 ± 0.8	40.3 ± 1.1	**7.87E-14**	39.5 ± 0.8	**9.23E-10**
Glucagon(f) (nG/L)	63.0 ± 4.5	60.0 ± 9.7	*0.177*	61.1 ± 3.6	*0.983*	62.5 ± 3.2	58.5 ± 4.0	0.242539	64.8 ± 5.5	0.522
AUC Glucagon (nG/L/3h)	541 ± 142	838 ± 337	*0.548*	3646 ± 546	**9.34E-11 #**	1114 ± 208	3536 ± 566	**1.56E-07**	671 ± 237	0.318#
Peak Glucagon (nG/L)	72.7 ± 5.8	70.2 ± 10.9	*0.470*	103 ± 11.2	**1.52E-05 #**	79.8 ± 4.3	100.5 ± 6.8*	0.006471*	77.3 ± 7.3	0.767

Values are means±SEM. Bold p-values: p<0,00037 within groups before and after intervention; # p<0,00037 significant different effect of different interventions between either DM groups or NGT groups. A trend (not significant after correction) was defined as p<0,05 and marked by *. Abbreviations: BMI: body mass index; FFA: free fatty acids; NGT: normal glucose tolerant; T2DM: type 2 diabetes mellitus; AUC: area under the response curve (as calculated from fasting level), GLP-1: glucagon-like-peptide-1; GIP: gastric inhibitory peptide.

Postprandial

AUC glucagon was unaffected by the VLCD or GB, but in contrast, increased after RYGB in NGT (p=9.34E-11) and T2DM (p=1.65E-7) subjects (table 2, figure 1). There was a downward shift of the glucose curve after VLCD in T2DM subjects (14). AUC insulin increased after RYGB in NGT and T2DM subjects and showed a left ward shift of the response curve and *decreased* after GB in NGT and VLCD in T2DM subjects (14).

Correlations

Multiple regression analysis was performed to correlate AUC of glucagon with glucose, insulin, GIP and GLP-1. A trend towards a positive correlation between AUC glucose with respectively AUC insulin (r=0.259; p= 0.035) and AUC glucagon (r=0.263; p=0.036), and of AUC glucagon with AUC GIP (r=0.278; p=0.026) was observed at baseline.

After RYGB there was a significant correlation between AUC GLP-1 and AUC Glucagon (r=0.543; p=0.0016), whereas no such correlation was found after VLCD/GB.

DISCUSSION

Here, we confirm that the fasting glucagon concentration is elevated in obesity, irrespective of the presence of T2DM. In obesity with T2DM, postprandial suppression of glucagon release is clearly inadequate as well. In view of the physiologic effects of glucagon (4), these anomalies may contribute to the metabolic disorder in these patients (4;9). However, three weeks after Roux-en-Y gastric bypass, the postprandial rise of glucagon in T2DM (and NGT) was even higher than before the intervention. The same effect was seen in obese individuals without diabetes. Moreover, we observed a correlation between the postprandial responses of glucagon and GLP-1. Despite the increase in glucagon, glucose metabolism was clearly improved, particularly in diabetic subjects. In contrast, gastric banding or a VLCD did not affect glucagon levels, neither in fasting condition nor in response to a meal, while either intervention ameliorated glucose metabolism as well as RYGB.

In healthy individuals, the glucagon-inhibiting effects of GLP-1, insulin and glucose outweigh the postprandial glucagonotropic effects of GIP and GLP-2. Inadequate postprandial suppression of glucagon secretion in T2DM could thus be caused by increased glucagonotropic effects of GIP/GLP-2 or by resistance to the glucagonostatic effects of GLP-1, insulin or glucose. The glucagonostatic effect of GLP-1 and glucose appear to be preserved in T2DM (15;16), but alpha-cells are probably resistant to insulin

(17). In our study, the postprandial plasma GIP levels were higher in T2DM subjects and tended to correlate with postprandial glucagon concentrations. Thus, the concerted effects of increased GIP release and insulin resistance of α-cells may drive postprandial hyperglucagonemia in type 2 diabetes.

Postprandial glucagon levels were increased even further shortly after RYGB, when weight loss was still minimal. In contrast, neither VLCD nor GB affected postprandial glucagon release. These findings suggest that the gastrointestinal rearrangements of RYGB, but not calorie restriction, cause glucagon levels to rise in response to a meal. Interestingly, we also show a positive correlation between the increase in postprandial GLP-1 and glucagon levels after RYGB, which was not shown before (13;18). GLP-1 normally inhibits glucagon release (4). Apparently, other cues overrule the glucagonostatic effects of GLP-1 (and insulin) after RYGB, and thereby induce this paradoxical effect. Possibly, nutrient-induced glucagon release may be reinforced in the context of more rapid entry of nutritional secretagogues into the portohepaticcirculation after duodenal bypass (12), thereby activating portal glucose sensors. Portal hyperglycemia shifts glucose uptake towards the liver and away from other peripheral tissues such as adipose tissue and muscle (19), thereby inducing glucagon secretion by alpha cells (20)**.** Increased glucagon release by the gut in response to food intake, in concert with GLP-1, may be an alternative explanation for postprandial hyperglucagonemia after RYGB. Physiologically, both glucagon and GLP-1 share the same prohormone, proglucagon, which is processed to GLP-1 and glucagon by prohormoneconvertase (PC) 1 in the gut and PC2 in pancreatic alpha cells respectively (21). However, PC2, next to PC1 is expressed in the proximal gut (22), and therefore might be affected by altered gut stimulation after RYGB.

In view of the well known stimulatory effect of glucagon on glucose production (4;23), it is unlikely that hyperglucagonemia contributes to the early metabolic beneficial effects of RYGB. However, glucagon also inhibits food intake in rodents and humans, it stimulates energy expenditure and fatty acid oxidation and lowers circulating lipids (24). Therefore, the effects of glucagon may be tissue specific and as such beneficial to whole body energy metabolism. Indeed, a dual glucagon/GLP-1 receptor agonist was reported to have superior lipid- and bodyweight-lowering effects as compared to a selective GLP-1 receptor agonist in diet induced obese mice (25). Therefore, the simultaneous increase of postprandial GLP-1 and glucagon levels may well contribute to the (long term) effects of this procedure on bodyweight and concomitant metabolic benefits.

In conclusion, we show that fasting plasma glucagon levels are similarly elevated in obese humans, with or without T2DM. The postprandial rise of glucagon is clearly higher in diabetic individuals. We also demonstrate that RYGB, but not GB or calorie restriction, increases postprandial glucagon concentration even further in obese humans, irrespective of the presence of T2DM. These data suggest that the gastrointestinal rearrangements of RYGB are responsible for the rise of postprandial glucagon levels. Although hyperglucagonemia is unlikely to contribute to the early glucose lowering effects of RYGB, the concerted effects of high postprandial GLP-1 and glucagon concentrations may well drive long term weight loss and concomitant metabolic benefits in response to this procedure.

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