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BRIEF COMMUNICATION

No Islet Cell Hyperfunction, but Altered Gut-Islet Regulation and Postprandial Hypoglycemia in Glucose-Tolerant Patients 3 Years After Gastric Bypass Surgery

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Abstract Postprandial hyperinsulinemia characterizes Roux-en-Y gastric bypass (RYGB) and sometimes leads to reactive hypoglycemia. We prospectively evaluated changes in beta cell function in seven RYGB-operated patients with a median follow-up of 2.9 years with hyperglycemic clamps and oral glucose tolerance tests (OGTTs). Three years after RYGB, weight loss was 26 % and insulin sensitivity had improved. Insulin secretion during clamp experiments was largely unchanged compared to before surgery. In contrast, insulin secretion in response to the OGTTs doubled when evaluated by the disposition index and 2-h plasma glucose declined to a mean of 3.3 ± 0.3 mmol/l postoperatively. Our findings indicate that intrinsic beta cell function remains unchanged in glucose-tolerant patients even years after RYGB, while altered gut-islet regulation drive risk of postprandial hyperinsulinemic hypoglycemia.

Keywords Bariatric surgery · Glucagon · Glucagon-like peptide-1 · Glucose-dependent insulinotropic polypeptide · Insulin secretion rate · Obesity · Insulin resistance

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Introduction

Roux-en-Y gastric bypass (RYGB) surgery has marked effects on glucose metabolism, particularly in the postprandial state that is characterized by accelerated glucose absorption, insulin hypersecretion, and exaggerated gut hormone release [1]. Although beneficial in patients with preoperative type 2 diabetes, these effects may also lead to an increased risk of late-onset hyperinsulinemic hypoglycemia [2].

We have previously reported changes in islet cell function thoroughly assessed by hyperglycemic clamps and oral glucose tolerance tests (OGTTs) before and up to 3 months after RYGB in a small cohort of patients with preoperative normal glucose tolerance (NGT) [3]. OGTTs resulted in insulin hypersecretion, low 2-h plasma glucose, and hyperglucagonemia, while islet cell responses during the clamps were unchanged, leading to the conclusion that postprandial hyperinsulinemia is related to the oral route of delivery. An important limitation, however, was the lack of long-term follow-up, since symptomatic hyperinsulinemic hypoglycemia usually develops years after surgery. For this reason, we decided to investigate the same parameters 3 years after RYGB.

Methods

Participants Seven of 11 patients from our previous study [3] accepted an invitation to participate in the follow-up study. One patient was excluded due to pregnancy and three patients declined our offer to participate. All patients had normal glucose tolerance evaluated by HbA1c and 2-h plasma glucose (Table 1). Additional comorbidities are listed in Supplementary Table 1. In the postoperative

Table 1 Characteristics and measures of insulin, glucagon, and hormonal responses to clamps and OGTTs in RYGB-operated patients and controls

	Patients				Controls	
	Before	3 months	3 years	*p* (time from surgery)		*p* (versus patients at 3 years)
Gender (m/f)	4/3				2/5	
Age (years)			47.5 ± 4.0		44.3 ± 3.2	0.543
Time from surgery (weeks)[a]	−1.6 (−3.1; −0.1)	13.9 (10.4; 15.1)	149.9 (112.9; 177.1)	NA		
BMI (kg/m^2)	43.2 ± 1.7	36.9 ± 1.6***	32.0 ± 1.0***††	<0.001	31.4 ± 1.0	0.664
Basal concentrations						
HbA1c (mmol/mol)[a]	34 (31; 41)	34 (27; 40)	31 (27; 38)	0.145	35 (30; 39)	0.482
Glucose (mmol/l)	5.5 ± 0.2	5.0 ± 0.1**	5.2 ± 0.2	0.015	5.4 ± 0.1	0.415
Insulin (pmol/l)[b]	132 ± 26	59 ± 9**	47 ± 3***	<0.001	82 ± 19	0.104
ISR (pmol kg^{-1} min^{-1})	2.81 ± 0.43	1.92 ± 0.24*	1.81 ± 0.09*	0.027	2.07 ± 0.14	0.149
Glucagon (pmol/l)[b]	8.3 ± 0.8	6.6 ± 0.7	6.9 ± 0.7	0.090	9.1 ± 1.0	0.165
GLP-1 (pmol/l)	NA	NA	11.0 ± 0.7	NA	9.9 ± 1.2	0.431
GIP (pmol/l)	NA	NA	11.0 ± 0.9	NA	8.4 ± 0.9	0.062
Insulin sensitivity						
1/HOMA-IR	0.60 ± 0.10	1.08 ± 0.19**	1.16 ± 0.08**	0.007	0.82 ± 0.15	0.099
OGIS (ml min^{-1} m^{-2})	344 ± 24	397 ± 12	459 ± 14***†	0.002	382 ± 20	0.010
Hyperglycemic clamp						
AIR_{glu} (pmol kg^{-1} min^{-1})[b]	10.9 ± 3.1	8.6 ± 1.5	9.3 ± 2.2	0.444	13.3 ± 2.2	0.141
Second phase (pmol kg^{-1} min^{-1})[b]	4.7 ± 1.5	3.9 ± 0.6	3.7 ± 0.9	0.772	3.8 ± 0.2	0.501
AIR_{arg} (pmol kg^{-1} min^{-1})[b]	20.4 ± 4.2	21.1 ± 4.2	20.3 ± 2.3	0.908	25.0 ± 2.6	0.199
$DI_{IV,HOMA}$[b]	4.32 ± 1.43	5.46 ± 1.12	6.89 ± 1.84	0.130	8.12 ± 1.62	0.359
$DI_{IV,OGIS}$[b]	3804 ± 1120	3421 ± 621	4373 ± 1099	0.502	5114 ± 878	0.409
$Glucagon_{clamp}$ (pmol/l)	5.1 ± 0.3	3.7 ± 0.5*	2.9 ± 0.6**	0.011	3.1 ± 0.9	0.798
$Glucagon_{supp}$ (%)	36.8 ± 5.0	43.1 ± 7.3	59.1 ± 7.8**	0.026	66.7 ± 7.8	0.499
2 h OGTT						
Glucose						
iAUC (mmol/l min)[b]	233 ± 52	187 ± 51	241 ± 70	0.469	185 ± 38	0.556
Peak (mmol/l)[b]	8.7 ± 0.7	9.0 ± 0.7	10.3 ± 1.0	0.101	8.5 ± 0.7	0.157
2-h glucose (mmol/l)	5.6 ± 0.4	3.5 ± 0.3***	3.3 ± 0.3***	<0.001	5.9 ± 0.3	<0.001
Insulin						
iAUC (nmol/l min)	55.8 ± 11.8	76.3 ± 13.7	63.9 ± 10.6	0.456	34.8 ± 8.3	0.053
Peak (pmol/l)[b]	903 ± 260	1252 ± 257	1242 ± 248	0.333	540 ± 98	0.020
ISR						
iAUC (pmol/kg)	865 ± 67	1234 ± 127*	1172 ± 132*	0.035	830 ± 151	0.115
Peak (pmol kg^{-1} min^{-1})	13.6 ± 1.6	19.8 ± 1.9*	21.5 ± 1.9**	0.008	12.8 ± 2.0	0.008
IGI (pmol kg^{-1} min^{-1} [mmol/l]$^{-1}$)[b]	3.36 ± 0.80	6.03 ± 1.44**	4.23 ± 0.77	0.007	3.38 ± 0.47	0.365
$DI_{oral,HOMA}$[b]	2.06 ± 0.65	6.59 ± 2.05***	4.82 ± 0.76**	0.001	2.74 ± 0.60	0.045
$DI_{oral,OGIS}$[b]	1194 ± 321	2430 ± 630**	1985 ± 400*	0.006	1301 ± 202	0.136

Mean ± SEM

AIR_{arg} acute insulin response to arginine, *AIR_{glu}* acute insulin response to glucose, *BMI* body mass index, *DI* disposition index, *GIP* glucose-dependent insulinotropic polypeptide, *GLP-1* glucagon-like peptide-1, *$Glucagon_{clamp}$* nadir glucagon concentration during the clamp, *$Glucagon_{supp}$* percent glucagon suppression during the clamp, *HbA1c* glycated hemoglobin, *HOMA-IR* homeostatic model assessment 2 of insulin resistance, *iAUC* incremental area under the curve, *IGI* insulinogenic index, *ISR* insulin secretion rate, *OGIS* oral glucose insulin sensitivity index, *OGTT* oral glucose tolerance test, *NA* not applicable, *RYGB* Roux-en-Y gastric bypass

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with before RYGB; †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$ compared with 3 months post-RYGB

[a] Median (range)

[b] Logarithmic transformation

follow-up period, none of the patients had been hospitalized or medically treated for dumping symptoms or episodes of severe hypoglycemia. Seven age- and body mass index (BMI)-matched healthy controls were recruited by announcement at www.forsoegsperson.dk.

Experimental Design On separate days in the morning after a 10–12-h fast, the participants underwent a 60-min hyperglycemic clamp and a 180-min OGTT. Identical tests had been performed before and 3 months after RYGB as previously reported [3], although at these visits OGTT duration was

120 min. For a detailed description of the experimental setup, please see supplementary material. During the clamps, mean ± SD plasma glucose level was 9.0 ± 0.3 mmol/l and CV ± SD was 4.3 ± 2.2 %. OGTTs were well tolerated, except for mild degrees of nausea and fatigue during the first hour after glucose ingestion in patients postoperatively.

Surgical Procedure Surgery was performed at the Department of Gastroenterology at Hvidovre Hospital (Hvidovre, Denmark) using a standard laparoscopic RYGB technique, resulting in a gastric pouch with a volume of about 25 ml, a 100-cm-long Roux-limb, and a 75-cm-long biliopancreatic limb.

Assays Blood samples were collected and stored as previously described [3]. All samples from before and 3 months after surgery were re-analyzed together with samples from the follow-up study to minimize assay aberrations, except for plasma samples from the OGTTs that were not available. Plasma samples for glucose, glucagon and total glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) were analyzed as previously described [3], and serum insulin and C-peptide concentrations were determined by Immulite 2000 analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

Calculations and Statistical Analysis Insulin sensitivity was assessed based on the reciprocal of HOMA-IR and the oral glucose insulin sensitivity (OGIS) index [3]. Incremental AUCs (iAUCs) were calculated using the trapezoidal rule subtracting basal levels. Prehepatic insulin secretion rate (ISR) was calculated from C-peptide using the ISEC software program [4]. Based on the OGTTs, the insulinogenic index (IGI) was calculated as $(ISR_{30\ min} - ISR_{basal})/(glucose_{30\ min} - glucose_{basal})$. From the clamps, three different indices of insulin secretion were calculated: (1) the acute insulin response to glucose (AIR_{glu}) as the mean increment in ISR above basal levels during the first 10 min of the clamp, (2) the second-phase insulin response as the mean increment ISR above basal levels from 20 to 40 min during the clamp, and (3) the acute insulin response to arginine (AIR_{arg}) as mean ISR in the first 5 min after injection of arginine subtracting the ISR level just prior to the injection (time 44 min). Insulin secretion in response to oral (IGI) and i.v. glucose (AIR_{glu}) was evaluated in relation to insulin sensitivity (1/HOMA-IR and OGIS) by calculating four disposition indices ($DI_{oral,HOMA}$, $DI_{oral,OGIS}$, $DI_{IV,HOMA}$, $DI_{IV,OGIS}$). Glucagon suppression during the hyperglycemic clamps ($glucagon_{supp}$) was calculated as the difference between basal and clamp glucagon concentration at 30 min, expressed as percentage of basal.

Changes in outcome measures were tested using linear mixed-effects models with time from surgery as fixed effect and individual participants as random effect. Logarithmic transformation was used if the distribution was skewed. Unpaired *t* test was used to compare patients at 3 years versus controls. $p < 0.05$ was considered significant. All analyses were performed in R version 2.13.0 (www.R-project.org).

Results

Baseline characteristics and measures of insulin, glucagon, and hormonal responses to clamps and OGTTs are presented in Table 1 and Fig. 1. By 3 years, BMI was reduced by 11.3 ± 1.5 kg/m^2 (total weight loss 25.6 ± 2.5 %). In the fasting state, glucose and glucagon concentration were unchanged, while insulin level and ISR declined gradually after surgery. Both 1/HOMA-IR and OGIS improved postoperatively. Except for OGIS that was higher at 3 years in patients, none of the basal parameters differed significantly between patients and controls.

Hyperglycemic Clamp (Fig. 1a–c) Up to 3 years after surgery, all indices of insulin secretion, including $DI_{IV,HOMA}$ and $DI_{IV,OGIS}$, were statistically unchanged and did not differ from controls. Glucagon concentrations were suppressed in response to i.v. glucose before and after surgery with somewhat lower nadir concentrations postoperatively and did not differ from controls.

OGTT (Fig. 1d–i) After RYGB, $iAUC_{glucose}$ and peak glucose concentration were unchanged, while 2-h plasma glucose was reduced and lower than in controls by 3 years. Six of seven patients had a postoperative 2-h plasma glucose <3.9 mmol/l compared to none before surgery, and in two patients, the 2-h plasma glucose was <2.8 mmol/l, although not associated with neuroglycopenic symptoms. While peripheral insulin concentrations were statistically unchanged after surgery, iAUC and peak concentrations of ISR increased. $DI_{oral,HOMA}$ and $DI_{oral,OGIS}$ were significantly elevated at both postoperative time points. Overall, 3 years after RYGB, insulin secretion was also increased compared to controls and postprandial GLP-1 secretion was elevated ($iAUC_{GLP\text{-}1}$ 9.1 ± 0.9 nmol min vs. 1.8 ± 0.3, $p < 0.001$), whereas GIP levels did not differ ($iAUC_{GIP}$ $p = 0.865$). Glucagon concentrations decreased in response to the oral glucose load in controls, whereas hyperglucagonemia was seen in patients ($iAUC_{Glucagon}$ $p = 0.004$). Similar results for patients vs. controls were seen for the full 3-h OGTT sampling period (Supplementary Table 2).

Discussion

The present study was conducted to prospectively evaluate long-term changes in islet cell function in glucose-tolerant

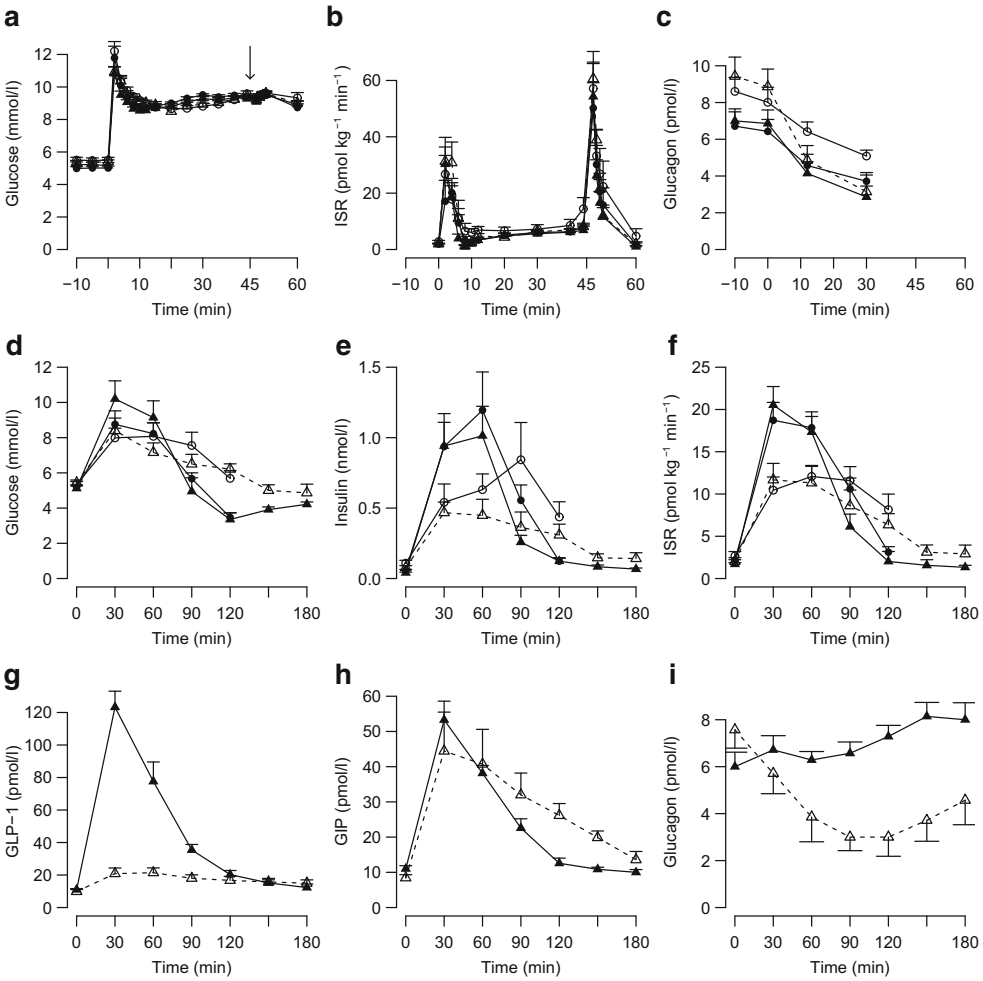

Fig. 1 Glucose (**a**), insulin secretion rate (ISR) (**b**), and glucagon (**c**) during hyperglycemic clamps. Glucose (**d**), insulin (**e**), ISR (**f**), glucagon-like peptide-1 (**g**), glucose-dependent insulinotropic polypeptide (**h**), and glucagon (**i**) during oral glucose tolerance tests. *White circles*/*solid line* before Roux-en-Y gastric bypass (RYGB), *black circles*/*solid line* 3 months after RYGB, *black triangles*/*solid line* 3 years after RYGB, *white triangles*/*dashed line* = controls, *Arrow* arginine bolus

patients after gastric bypass surgery. Median follow-up time was 2.9 years, and total weight loss was ~26 %.

The main finding was a largely unchanged postoperative insulin secretion during the clamp experiments throughout the entire follow-up period, even after adjusting for the improved insulin sensitivity (measured by OGIS and 1/HOMA-IR). This finding was further strengthened by the observation that 3 years postoperatively, all clamp-derived measures of insulin secretion were numerically lower in patients than in their age- and BMI-matched controls. To our knowledge, the present study represents the most comprehensive prospective long-term evaluation of beta cell function in glucose-tolerant patients after RYGB. Yet, our results are in line with shorter-term studies based on intravenous glucose tolerance tests (IVGTTs) [5, 6] and glucose-glucagon tests [7]. Further, a cross-sectional study found IVGTT-associated insulin secretion 4 years after RYGB to be comparable to matched morbidly obese controls [8].

In contrast to the unaltered intrinsic beta-cell function during the clamp experiments, we found an increased postoperative insulin secretion in response to the OGTTs, including a doubling of DI_{oral}. GLP-1 secretion was fivefold elevated compared to controls, in agreement with studies linking excess postoperative GLP-1 secretion with postprandial hyperinsulinemia [9, 10]. Interestingly, the most dramatic changes in response to the OGTT occurred already within the first 3 months after surgery with limited additional change at 3 years.

The postprandial hyperglucagonemia after RYGB observed in our study remains a puzzling finding, but is not explained by an altered alpha cell response to glucose since glucagon concentrations were adequately suppressed during the clamp experiments as also reported previously [10].

A limitation of the present study is the small size with only seven individuals in each group, which, however, is partly accounted for by the prospective design. Nevertheless, unpaired comparisons between groups are not sufficiently powered to rule out potentially important differences. Another limitation is that none of our patients, despite developing postprandial hypoglycemia postoperatively, experienced neuroglycopenic symptoms, which, however, is not surprising given the prospective design of our study and the low incidence of this condition [2]. Finally, insulin sensitivity was measured

indirectly using surrogate markers (i.e., 1/HOMA-IR and OGIS), which limits the accuracy of this measure, although we have found that both makers show an acceptable correlation with direct measures of insulin sensitivity obtained by the hyperinsulinemic euglycemic clamp [11].

In conclusion, our study adds comprehensive prospective long-term data demonstrating unaltered intrinsic islet cell function after RYGB in patients with preoperative NGT, despite dramatic changes in postprandial glucose tolerance with lower 2-h plasma glucose, hyperinsulinemia, hyperglucagonemia, and exaggerated GLP-1.

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Compliance with Ethical Standards

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Conflict of Interest CD has received a speaker honorarium from Eli Lilly. JJH has received grants from Novartis and Merck and is an advisory board member/consultant for GlaxoSmithKline, Novo Nordisk, Zealand Pharmaceuticals, AstraZeneca, Sanofi, MSD, Intarcia, and Hamni. AE, KBM, MS, CM, NBJ, and SM declare that they have no conflict of interest associated with this manuscript.

Ethical Approval The study conforms with the Declaration of Helsinki II and was approved by the Municipal Ethical Committee of Copenhagen (H-2-2014-011) and the Danish Data Protection Agency, and was registered at clinicaltrials.gov (NCT02161666).

Informed Consent Written informed consent was obtained from all participants in the study.

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