ORIGINAL ARTICLE

Beta-cell sensitivity to insulinotropic gut hormones is reduced after gastric bypass surgery

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ABSTRACT

Objective Postprandial hyperinsulinaemia after Roux-en Y gastric bypass (GB) has been attributed to rapid nutrient flux from the gut, and an enhanced incretin effect. However, it is unclear whether surgery changes islet cell responsiveness to regulatory factors. This study tested the hypothesis that β-cell sensitivity to glucagon like-peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) is attenuated after GB.

Design Ten non-diabetic subjects with GB, and 9 body mass index (BMI)-matched and age-matched non-surgical controls (CN) with normal glucose tolerance had blood glucose clamped at ~7.8 mM on three separate days. Stepwise incremental infusions of GLP-1 (15, 30, 60, 120 and 300 ng/LBkg/h), GIP (75, 150, 300, 600 and 1200 ng/LBkg/h) or saline were administered from 90 to 240 min and insulin secretion measured.

Results GB subjects had similar fasting glucose levels but lower fasting insulin compared with CN, likely due to increased insulin clearance. The average insulin secretion rates (ISRs) to 7.8 mM glucose were ~30% lower in GB relative to CN subjects. However, incretin-stimulated ISRs, adjusted for insulin sensitivity and glucose-stimulated insulin secretion, were even more attenuated in the GB subjects, by threefold to fourfold ($AUC_{ISR(90–240\ min)}$ during GLP-1 and GIP: 47±8 and 44±12 nmol in GB and 116±16 and 161±44 in CN; p<0.01).

Conclusion After GB, the sensitivity of insulin secretion to both glucose and incretins is diminished.

Significance of this study

What is already known about this subject?
- Weight-loss-independent effects of gastric bypass (GB) surgery on β-cell function is attributed to both rapid nutrient emptying from stomach pouch to the gut and an increased incretin effect.
- After GB, despite massive postprandial secretion of glucagon like-peptide 1 (GLP-1) (>10-fold), the GLP-1-stimulated insulin response is only modestly (2-fold) increased, suggesting reduced β-cell sensitivity to this peptide.
- Previous reports indicate that non-diabetic individuals with GB have impaired β-cell sensitivity to glucose.

What are the new findings?
- Beta-cell sensitivity to the incretins, GLP-1 and glucose-dependent insulinotropic peptide is reduced after GB.
- Impaired insulin secretory response to these gut peptides is independent of insulin sensitivity.

How might it impact on clinical practice in the foreseeable future?
- Decreased β-cell sensitivity to insulinotropic gut peptides several years after GB may represent adaptation of the enteroinsular axis to enhanced nutrient flux and incretin secretion, protecting against hypoglycaemia.
- Better understanding of long-term effect of GB on islet function can inform therapeutic options that are needed to address hyperglycaemia in the setting of rerouted gastrointestinal tract.

INTRODUCTION

Bariatric surgery is an increasingly used treatment for obese patients with diabetes.[1] Following gastric bypass (GB) or sleeve gastrectomy, obese patients with type 2 diabetes (T2DM) have high rates of remission that often last for many years.[1] Patients with GB[1] or sleeve gastrectomy[2] have improved insulin sensitivity as a result of weight loss, and those with T2DM have rapid improvement of glucose tolerance and insulin secretion,[3–5] often prior to the majority of weight loss. Understanding the mechanisms by which these changes occur has been the focus of considerable effort in recent years because the effect size of surgery far exceeds conventional treatments for diabetes.

Following GB enteral glucose flux is substantially enhanced, leading to rapid increases of meal glucose appearance in the circulation.[6–10] This rapid, elevated peak of plasma glucose is associated with early prandial hyperinsulinaemia, likely not only due to greater glucose stimulation of β-cells,[11] but also by an enhanced incretin effect.[12–14] In fact, one of the hallmarks of GB is a 10-fold increase in meal-induced secretion of glucagon-like peptide 1 (GLP-1),[15] a phenomenon that has been proposed to have a major influence on the insulin response in surgical patients.[16]

In a previous study using GLP-1 receptor blockade during meals, we observed that non-diabetic subjects with GB had twofold to threefold greater GLP-1-stimulated insulin secretion than control subjects matched for age, weight and gender.[14] However, the magnitude of the increased GLP-1 effect was not commensurate with the increase in prandial GLP-1 concentrations, which were elevated >10-fold compared with controls.[8]

These findings raise the possibility that β-cell sensitivity to GLP-1 is altered after bariatric surgery. We recently observed that β-cell sensitivity to graded doses of intravenous glucose is diminished in non-diabetic individuals after GB compared with matched controls without surgery.[17] Thus, we hypothesised that subjects with GB would also have reduced insulin responses to both exogenous GLP-1 and glucose-dependent insulinotropic polypeptide (GIP), and tested this in non-diabetic GB subjects and non-surgical controls.

METHODS

Subjects

Ten patients with a history of GB (GB) and 9 age-matched and BMI-matched non-diabetic subjects with no history of gastrointestinal (GI) surgeries (CN) were recruited consecutively in order of their presentation to the advertisement or to the clinic. None of the subjects had active medical conditions including diabetes, renal dysfunction or liver disorders and none were taking medications that interfere with glucose metabolism.

The surgical subjects were recruited at least 2 years after their surgery. They were weight stable for at least 3 months prior to enrolment, had no history of T2DM prior to surgery (no history of either hyperglycaemia or taking antidiabetic medications) and had lost an average of 59±8 kg (ranging from 3 to 86 kg) of body weight in an average of 5±1 years (ranging from 2 to 11 years) since surgery. The CN subjects had no personal or family history of diabetes, and normal glucose tolerance was confirmed by a 2-hour plasma glucose level of <7.8 mmol/L following 75 g oral glucose tolerance test.

All participants provided written informed consent before the studies.

Peptides

Synthetic human GLP-1-(7-36) amide and human GIP-(1-42) (Clinalfa, Bachem Distribution Services, Germany) were >95% pure, sterile and free of pyrogens. Lyophilised peptides were prepared in 0.25% human serum albumin on the day of study. The use of synthetic GLP-1 and GIP is approved under the US Food and Drug Administration INDs 32 977 and 110 035.

Experimental protocols

Participants were instructed to refrain from strenuous physical activity and maintain normal carbohydrate ingestion for 3 days before each visit. Study subjects were admitted to the General Clinical Research Center at Cincinnati Children's Hospital after an overnight fast in the morning. Body composition including lean body mass (LB) was assessed using dual-energy X-ray absorptiometry, and waist circumference was measured by a research dietician using a standardised method. Intravenous catheters were placed in each forearm for the withdrawal of blood and the infusion of 20% glucose, GLP-1 or GIP; the arm used for blood sampling was continuously warmed to maintain blood flow.

Each subject was studied on three occasions separated by at least 1 week. After the withdrawal of fasting blood samples, at 0 min, a primed continuous infusion of 20% glucose was started to achieve and maintain a target blood glucose level of 7.8 mmol/L for 300 min. This level of glycaemia approximates peak postprandial glucose peak in normal glucose-tolerant subjects during an oral glucose tolerance test. At 90 min, subjects received either a stepped infusion of GLP-1, GIP or saline, which continued until 240 min (see the online supplementary figure 1). Doses of peptides were calculated based on LB. GLP-1 infusion was started at 15 ng/LBkg/h, followed by infusions of 30, 60, 120 and 300 ng/LBkg/h; GIP infusion was started at 75 ng/LBkg/h and followed by infusion of 150, 300, 600 and 1200 ng/LBkg/h (see the online supplementary figure 1). Each peptide infusion rate was administered for 30 min. Plasma was separated within 60 min for storage at −80° C until assay.

Assays

Blood samples were collected in tubes containing heparin for determinations of plasma insulin and blood glucose. Blood was also collected in tubes containing 50 mM EDTA plus 500 kallikrein inhibitory units/mL aprotinin for the measurement of plasma C-peptide, GLP-1 and GIP and 0.1 M diprotin A for the measurement of active GLP-1. Blood glucose concentrations were determined at the bedside using an automated glucose analyser (YSI 2300 STAT Plus, Yellow Springs, Ohio, USA). Insulin concentrations were determined using commercial ELISA (Alpco, Salem NH), and concentrations of C-peptide by commercial radioimmunoassay (Millipore, Billerica, Massachusetts, USA), each according to the manufacturers' specifications. Total and active GLP-1 and GIP were assayed using a commercially available ELISA (MSD ELISA). Dipeptidyl peptidase- 4 (DPP-4) activity was assessed using a standard colorimetric assay.[18]

Calculations and analysis

The stability of hyperglycaemic clamps was computed as the average coefficient of variation (CVs) of blood glucose concentrations for each study from 30 to 300 min (the entirety of the clamp). Intrasubject comparisons of clamp stability were computed as the CV of average blood glucose concentrations for 30–300 min from the three studies in each subject.

Fasting values of blood glucose, heart rates and hormones were computed as the average of three measures from −15 to 0 min. Insulin secretion rates (ISR) were derived from plasma C-peptide concentrations using deconvolution with population estimates of C-peptide[19]; an assumption underlying this analysis is that GB does not affect C-peptide kinetics.

Mean, SD and SEM were computed for glucose, glucose infusion rate (GIR), insulin, ISR and heart rates from 70 to 90 min (steady-state glycaemia, before peptide infusion) and 90–240 min (hyperglycaemia plus peptide infusion). In addition, insulin, ISR, GLP-1 and GIP values during peptide infusions were used to calculate incremental areas under the curve (AUC) using the trapezoidal rule and are presented for times 90–240 min.

Insulin sensitivity was calculated during each study as the ratio of M/I (average GIR from 70 to 90 min divided by corresponding average insulin levels). Systemic insulin clearance was calculated by dividing the AUC_{ISR} by $AUC_{insulin}$ over the interval 0–240 min during three studies in each subject. Disposition index was measured as a product of insulin sensitivity (M/I) times acute insulin response ($AUC_{Insulin(0–10min)}$).

Table 1 Baseline characteristics of study participants

Baseline characteristics	GB (10)	CN (9)
Age (years)	41.4±2.2	41.9±3.1
BMI (kg/m^2)	32.6±1.7	32.3±1.4
Lean mass (kg)	51.5±2.4	60.2±4.3
Fat mass (kg)	35.5±4.5	31.2±2.7
Waist circumference (cm)	100.3±3.1	105.7±3.5
Sex (M/F)	1/9	3/6
A1C (% (mmol/mol))	5.0±0.1 (31±2)	5.1±0.2 (32±2)

Data are presented as mean±SEM unless specified otherwise.
BMI, body mass index; CN, non-surgical controls; GB, patients with gastric bypass.

Table 2 Blood glucose, glucose infusion rates and β-cell response to stepwise incremental infusions of GLP-1 or GIP or saline during hyperglycaemic clamp as well as fasting GLP-1 and GIP in subjects with a history of gastric bypass (GB) and non-surgical controls (CN)

	GLP-1		GIP		Saline		Statistical tests (P value)		
	CN	GB	CN	GB	CN	GB	Study day	Group	Day*Group
Fasting glucose (mmol/L)	4.6±0.1	4.3±0.1	4.5±0.1	4.3±0.1	4.6±0.1	4.4±0.1	0.715	0.302	0.716
GIR $_{(70–90min)}$ (mg/kg/min)	4.0±0.5	3.4±0.3	4.4±0.6	3.6±0.5	4.7±0.7	3.5±0.3	0.460	0.204	0.657
GIR $_{(90–240min)}$ (mg/kg/min)	9.7±1.3	8.0±0.8	11.1±1.2	8.6±1.0	7.0±0.9	4.8±0.4	**0.000**	0.732	0.114
Fasting insulin (pmol/L)	87.2.±28.2	43.5±7.3	89.6±23.0	40.6±6.2	72.8±11.8	36.2±3.8	0.579	**0.042**	0.725
Average insulin $_{(70–90min)}$ (pmol/L)	334.0±71.8	90.7±9.8	326.6±77.7	95.7±17.5	355.3±58.5	106.5±15.1	0.777	**0.001**	0.958
AUC $_{Insulin(90-240min)}$ (nmol/L/min)	184.7±32.5	32.9±5.4	204.8±51.4	30.8±5.3	7.6±3.1	1.5±0.6	0.000	0.000	**0.000**
Fasting ISR (pmol/min)	119.2±14.7	84.2±6.1	135.6±15.9	105.3±9.2	124.5±13.9	98.6±7.9	**0.008**	0.055	0.821
Average ISR $_{(70–90min)}$ (pmol)	442.7±41.5	293.7±19.4	491.0±43.4	368.6±35.4	469.3±54.2	343.6±33.3	0.148	**0.010**	0.885
AUC $_{ISR(90–240min)}$ (nmol)	116.4±16.0	46.5±8.4	160.5±44.4	44.3±12.4	3.5±1.7	1.8±0.7	0.000	0.004	**0.007**
Insulin sensitivity (M/I) $_{(70–90min)}$	0.1±0.0	0.3±0.1	0.1±0.0	0.3±0.1	0.1±0.0	0.3±0.0	0.357	0.950	**0.002**
Insulin clearance $_{(0–240min)}$	0.9±0.1	2.4±0.2	1.0±0.1	2.9±0.4	1.3±0.2	4.2±0.6	**0.003**	**0.000**	0.169
Disposition index (AUC $_{Insulin(10min)}$X M/I)	286±65	292±40	250±49	238±42	285±65	257±32	0.492	0.950	0.305
Fasting GLP-1 (pg/mL)	8.1±1.5	7.0±1.0	6.0±1.2	7.0±1.0	8.9±1.8	6.3±1.0	0.314	0.150	0.638
Fasting GIP (pg/mL)	76.3±34.7	52.3±7.0	72.6±30.2	60.1±9.4	60.3±16.9	55.1±8.5	0.250	0.620	0.348

Data are presented as mean±SEM.
The significant P values are bolded
Statistical main effects of study day (GLP-1/GIP/saline) and group status (GB/CN) as well as their interaction (Day*Group) using a two-way repeated measures analysis of variance are provided in the last three right-hand columns.
AUC, incremental areas under the curve; GIR, glucose infusion rate; GIP, glucose -dependent insulinotropic peptide; GLP-1, glucagon like-peptide 1; ISR, insulin secretion rate.

Statistical analysis

Data are presented as mean±SEM. Baseline characteristics listed in table 1 were compared using analysis of variance (ANOVA) or χ^2 test. The parameters listed in table 2 obtained from each subject among the three studies were compared using a two-way repeated measures ANOVA. In addition, ISRs during the clamps with a peptide infusion study were compared between GB and CN subjects using ANCOVA adjusted for the covariance of M/I, and ISR during the saline clamp to adjust for insulin sensitivity and within-subject variation. Statistical analyses were performed using SPSS 25.

RESULTS

Subjects

The two groups of GB and CN were tightly matched for age and BMI; body composition and waist circumference were comparable and did not differ statistically (table 1). Both groups had a larger ratio of females to males, typical of background cohorts having bariatric surgery,[20] and glycated haemoglobin (HbA1C) levels were similar in both groups (table 1).

Glucose disposition during hyperglycaemic clamp

Fasting glucose levels were comparable for each subject among the three studies and among both surgical and non-surgical groups (figure 1B, table 2). For all experiments, blood glucose was raised to the glycaemic target (~7.8 mmol/L) at a comparable rate and maintained until completion of the study. The mean CV for blood glucose values for the duration of the fixed hyperglycaemia (30–300 min) were 3.4±0.5, 3.7±0.3 and 2.1±0.3% in the GB subjects during GLP-1, GIP and saline clamp studies, respectively, compared with 3.8±0.6, 4.3±0.6 and 4.2±0.5% in the CN individuals for the corresponding studies. The average glucose levels from 30 to 300 min were identical among the three clamp studies and between the groups (CV: 0.9±0.1% in CN vs 0.8±0.2% in GB).

The amount of glucose infusion needed to reach and maintain similar glycaemic levels (glucose disposition) during the first 90 min of the glucose clamp were similar between the GB and CN groups, and among the three study conditions (glucose disposition in GB subjects for GLP-1, GIP and saline studies: 420±28 vs. 449±38 mg/kg, 430±28 and in CN subjects: 442±40 mg/kg and 424±21 vs. 479±43 mg/kg, respectively).

Beta-cell response to hyperglycaemia, insulin clearance and insulin sensitivity

Fasting values of insulin and ISR were significantly lower in GB compared with CN subjects (table 2) while the ratio of fasting ISR over insulin levels, an estimate of basal insulin clearance, tended to be higher in the surgical subjects (CN: 2.1±0.4 and GB: 3.0±0.4). The insulin (see the online supplementary figure 2) and ISR values (figure 1) after intravenous glucose administration, both early phase insulin response (0–10 min) and second phase insulin secretion (70–90 min), were lower in GB subjects compared with non-surgical controls; this pattern of response did not differ among the three clamp studies (table 2).

Insulin clearance during the clamps was twofold to threefold greater in GB subjects compared with CN subjects (figure 2, table 2). Consequently, plasma insulin concentrations during the 70–90 min steady-state period were threefold to fourfold reduced, while ISR was only 30% lower (table 2). Given a similar steady state GIR, the estimated insulin sensitivity (M/I) was threefold higher in the GB subjects compared with CN subjects (table 2).

The relative increases in glucose-stimulated ISR (70–90 min) to fasting ISR were similar in the GB and CN groups and among the three studies (~3.8 vs ~3.6 in CN vs GB; table 2), despite group differences in the absolute ISR from 70 to 90 min. During the glucose clamp with saline, insulin secretion rates from 90 to 240 min did not change significantly compared with those from 70 to 90 min in either group (figures 1A and 3).

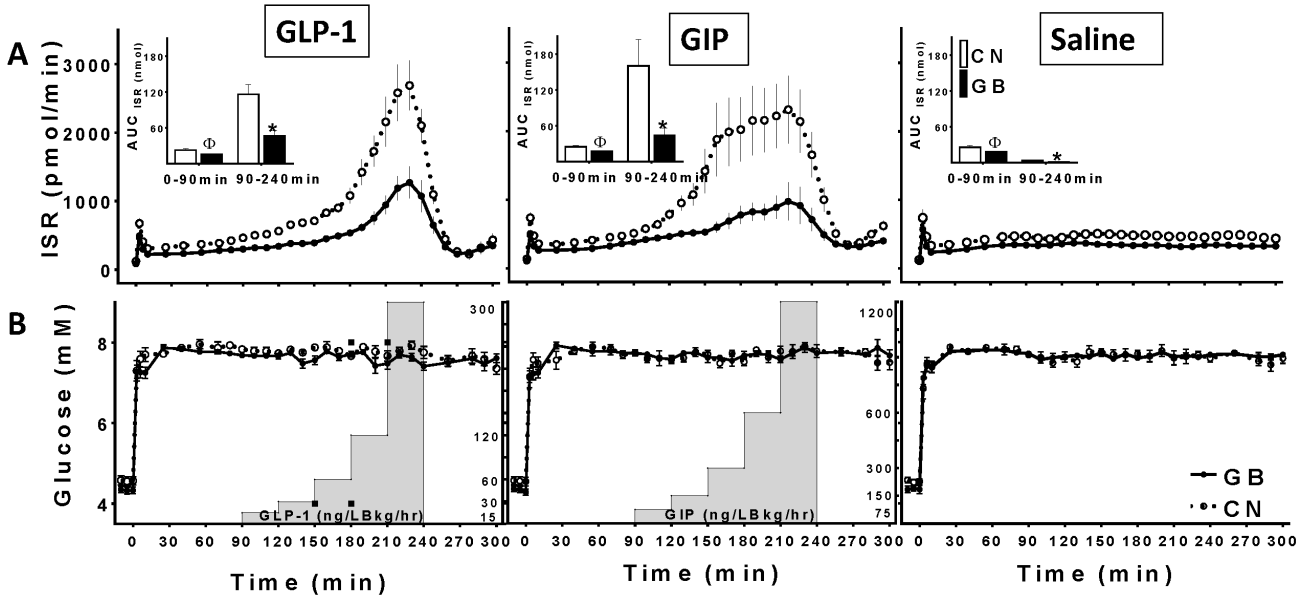

Figure 1 (A) Insuiln secretion rates and (B) blood glucose levels during hyperglycaemic clamp in subjects who underwent GB (GB, solid line and closed circle, black bar) and non-surgical controls (CN, dashed line and open circle, white bar). The corresponding areas under the curves for 90 to 240 min are shown (insets). *P<0.001 for interaction of the metabolic group and study days, ϕ p<0.05 compared with controls. Data are presented as mean±SEM.

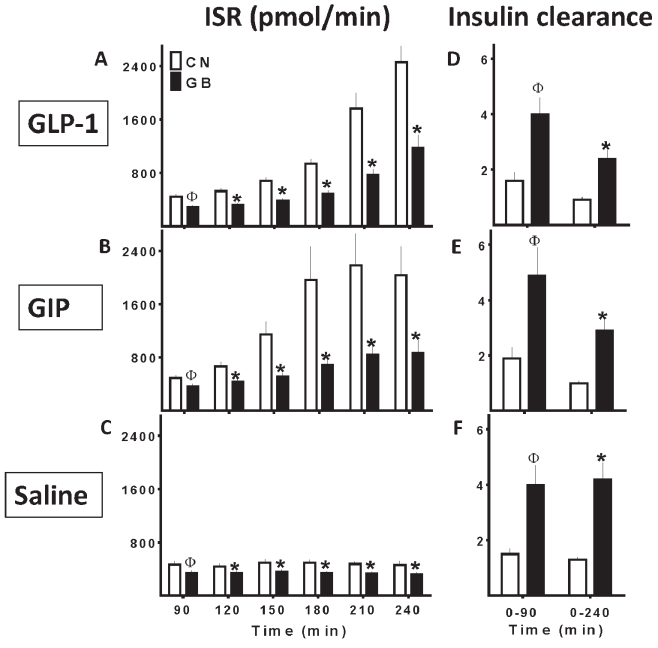

Figure 2 (A–C) Average insulin secretion rates calculated for stepwise infusion of increasing doses of (A) GLP-1, (B) GIP, and corresponding (C) saline studies during a hyperglycaemic clamp. (D–F) Insulin clearance calculated for 90 min (prior to peptide infusion) and 240 min during (D) GLP-1, (E) GIP and (F) saline studies. *P<0.05 for interaction of metabolic group and study days, ϕ p<0.05 compared with controls. Data are presented as mean±SEM. CN, non-surgical controls; GB, patients with a history of gastric bypass; GIP, glucose-dependent insulintropic peptide; GLP-1, glucagon like-peptide 1.

Insulin sensitivity (M/I) had a positive correlation with the acute insulin response to glucose infusion ($AUC_{Insulin(10min)}$) among the two groups (ρ:0.5, p<0.05), thus the disposition index was

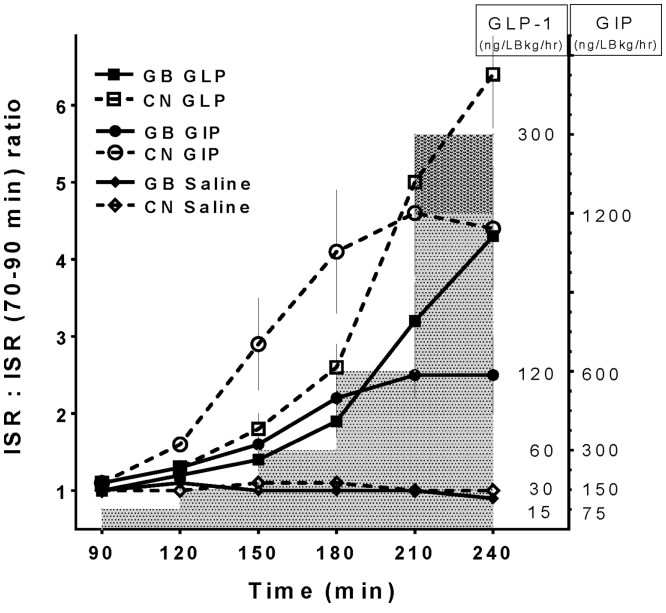

Figure 3 Incremental insulin secretory response to increasing doses of GLP-1 (square) and GIP (circle) compared with saline infusion (diamond) during hyperglycaemic clamp in subjects who had GB (GB, solid line) and non-surgical controls (CN, dashed line). Data are presented as mean±SEM. GIP, glucose-dependent insulintropic peptide; GLP-1, glucagon like-peptide 1.

similar between the two groups and among the three studies (table 2). However, there was no association between estimated M/I and incremental insulin levels from 90 to 240 min ($AUC_{Insulin(90-240min)}$) or insulin response from 70 to 90 min during saline studies.

Beta-cell responses to GLP-1 and GIP during fixed hyperglycaemia

In both GB and CN subjects, the stepwise increase of GLP-1 raised insulin secretion dose-dependently (figure 3). In contrast, the graded administration of GIP caused a plateau of ISR once the dose increased to 600 ng/LBM/h (figure 3). Incremental insulin levels (see the online supplementary figure 2) or ISR responses (figures 1A and 2A) to both GLP-1 or GIP infusion, estimated by $AUC_{Insulin(90-240min)}$ or $AUC_{ISR(90-240min)}$, were significantly reduced in GB subjects compared with CN ($p<0.01$ for interaction of subject group and study days) (table 2). When the incretin-stimulated ISR were adjusted for insulin sensitivity as well as ISR from the saline clamp using ANCOVA, the overall insulin secretory response to GLP-1 or GIP ($AUC_{ISR(90-240min)}$) remained substantially less in GB subjects during both GLP-1 and GIP infusion ($p<0.01$ for either peptide).

Infusion of GLP-1 and GIP led to significant incremental changes in the GIR necessary to maintain target glycaemia, and the relative increase of GIR was greater from 90 to 240 min during the peptide, compared with saline, infusions in both groups (table 2). The amounts of glucose infused from 90 to 240 min to maintain stable glycaemia were larger in CN individuals compared with GB subjects even after adjustment for insulin sensitivity (glucose disposition for GLP-1 and GIP studies in GB vs CN subjects: 1.2±0.1 and 1.3±0.1 g/kg vs 1.5±0.2 and 1.7±0.2 g/kg, respectively; $p<0.05$ adjusted for insulin sensitivity).

Beta-cell sensitivity to the incretins, estimated by the mean slopes of each individual's plot of ISR versus peptide concentration (total GLP-1 and GIP), was significantly smaller in GB subjects even after adjustment for insulin sensitivity [figure 4; GLP-1 sensitivity CN: 57.2±13.6, GB: 20.7±4.5 (pmol/min)/(pg/mL), $p=0.01$; GIP sensitivity CN: 1.5±0.3, GB: 0.5±0.2 (pmol/min)/(pg/mL), $p=0.01$]. The difference between the two groups was more pronounced when the slopes of ISR:GIP were calculated for 90–210 min before ISR plateaued. For each subject, the slopes of ISR versus GLP-1 and ISR versus GIP were significantly related (ρ:0.8, $p<0.0001$). Among the surgical subjects neither ISR: GLP-1 nor ISR: GIP slopes correlated with BMI, age, insulin sensitivity, and amount of weight loss or time since surgery.

GLP-1 and GIP and DPP-4 activity

Fasting plasma levels of total GLP-1 and GIP were similar in both groups and among the three studies and they did not differ between the two groups throughout the saline clamp study (table 2). Plasma levels of GIP were increased similarly in both groups in a linear fashion with the stepwise infusion of GIP based on LB ($AUC_{GIP(90-240min)}$: 51.0±3.4 vs 45.6±2.6 ng/mL/min in CN vs GB) (figure 5A). The average increase of total GLP-1 during the stepwise infusion followed a similar pattern ($AUC_{GLP-1(90-240min)}$: 1692±220 vs 2227±177 pg/mL/min in CN vs GB) although there was a trend for higher concentrations in the GB subjects compared with CN ($p=0.1$; figure 5B). Similarly, concentrations of active GLP-1 were lower in the CN than GB subjects during infusion of synthetic GLP-1 ($AUC_{GLP-1(7-36)[90-240min]}$: 682±277 vs 1422±180 pg/ml/min in CN vs GB, $p=0.06$; figure 4C).

DPP-4 activity at baseline was similar among the studies and between the surgical and non-surgical groups (see the online supplementary figure 3). Values measured at baseline during GLP-1 and GIP infusion were associated with those from 200 min (ρ: 0.5, $p<0.05$). Reproducibility of DPP-4 activity estimated as the coefficient variation of this parameter at baseline among three studies was 9±1% vs. 15±4% in GB vs. CN subjects.

The areas under the curve of active GLP-1 in response to GLP-1 infusion was significantly correlated to total GLP-1 AUC (ρ: 0.7, $p<0.01$), but no association was found between DDP-4 activity and active GLP-1 levels.

Heart rate and tolerance of peptides

Infusions of both GLP-1 and GIP were well tolerated. Fasting heart rates were similar among the surgical and non-surgical individuals and between the three clamp studies and remained relatively constant throughout the saline clamp study. The peptide infusions increased the heart rate similarly in both GB and CN groups by ~10% during the GLP-1 infusion and ~30% during infusion of GIP ($p<0.001$ for both peptides compared with the saline study); heart rate remained elevated despite discontinuation of GLP-1 and GIP during the last 60 min of the experiment ($p<0.001$ compared with control study) (see the online supplementary figure 4).

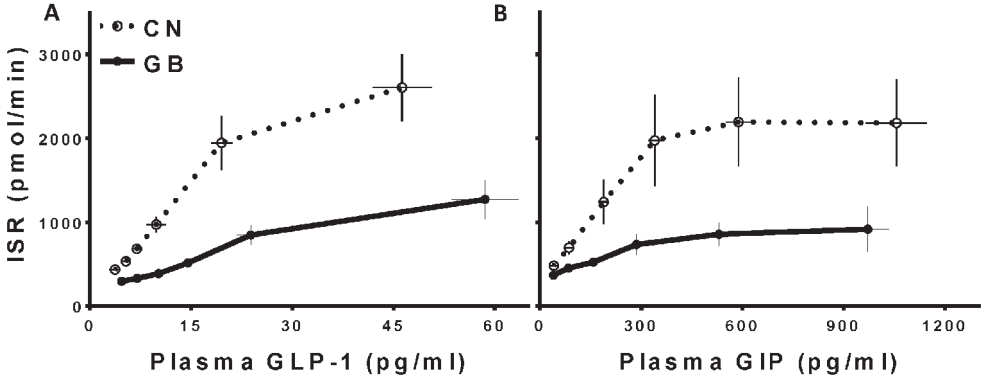

Figure 4 Slopes of ISR in response to (A) circulating levels of total GLP-1 and (B) GIP in subjects after GB (GB, solid line and closed circle) and non-operated healthy controls (CN, dashed line and open circles). Data are presented as mean±SEM. GIP, glucose-dependent insulintropic peptide; GLP-1, glucagon-like peptide 1; ISR, insulin secretion rate.

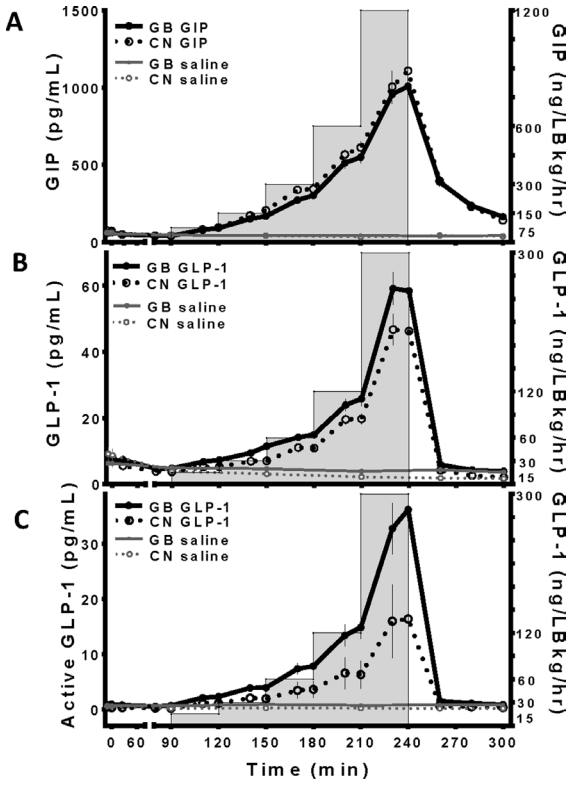

Figure 5 (A) Plasma GIP, (B,C) total and active GLP-1 levels during stepwise infusion of each peptide in subjects who underwent GB (GB, solid line and closed circle) and non-surgical controls (CN, dashed line and open circle). The corresponding plasma levels of each peptide are also shown during saline infusion in GB subject (solid grey line and closed circle) and CN subjects (dashed grey line and open circle). Data are presented as mean±SEM. GIP, glucose-dependent insulintropic peptide; GLP-1, glucagon-like peptide 1.

DISCUSSION

One of the hallmarks of GB is the rapid, accentuated rise in prandial blood glucose and accompanying hyperinsulinaemia.[6 7 10 11 15] Increased prandial insulin secretion after GB has been attributed in part to relative hyperglycaemia and the massive secretion of GLP-1,[15 21] with increased β-cell stimulation by GLP-1[14 22 23] and overall enhancement of the incretin effect.[12] However, the findings presented here are not compatible with this simple, but an inferential model. In our cohort of non-diabetic GB subjects, β-cell sensitivity to both GLP-1 and GIP is markedly reduced compared with the age-matched and BMI-matched group of subjects without intestinal surgery. These findings suggest that individuals chronically adapted to GB have muted β-cell responses to meal-induced stimuli like incretins, similar to what we have reported previously for glucose.[17]

We used a direct method of assessing β-cell sensitivity to GLP-1 and GIP, using graded infusions of the peptides[24] in the fasting state to eliminate confounding from the high rates of intestinal glucose appearance and incretin secretion that accompanies meal ingestion in GB. Plots of ISR versus GLP-1 concentration were generally linear among individuals allowing computation of a slope to represent the responses of individual subjects; despite the apparent plateau in ISR with GIP infusion, the estimated slopes from baseline to plateau or from the entire infusion period did not differ significantly, so we used the latter estimate among subjects in this study. Thus, the ramp protocol provides insulin:incretin dose–response during fixed hyperglycaemia that is independent of the many prandial inputs to insulin secretion. We conducted a glucose-only clamp to allow for within-subject adjustment for differences in insulin sensitivity and glucose-stimulated insulin secretion, increasing the statistical power of the incretin-stimulated insulin secretion comparisons. Finally, the derivation of ISR allowed the large effect of GB on insulin clearance to be clearly detected.

We used a cross-sectional design, which allowed us to evaluate the effects of GB beyond the first 2 years. Our subjects were recruited consecutively and with standard inclusion criteria including the lack of diagnosis of diabetes prior to surgery. While arguably a small cohort to be broadly representative of subjects with long-term GB, the participants in this project had no features to suggest an unusual β-cell phenotype *a priori*. A recent study evaluated the β-cell responsiveness to a single-dose infusion of GLP-1 or GIP during a 60 min hyperglycaemic clamp in normal glucose tolerant individuals before and 3 months after GB.[25] Insulin secretory responses to either GLP-1 or GIP in this setting remained unchanged within 3 months of surgery. Our subjects were fully adapted to surgery with maximal weight loss achieved and maintained at the time of the study. We infer from this distinction that β-cell adaptation to the physiological outcomes of weight loss surgery may take more than a few months after the procedure to develop.

The major finding reported here, reduced β-cell incretin sensitivity, was independent of blood glucose, insulin sensitivity or lean/fat mass. The diminished β-cell sensitivity to the incretins was evident across the range of GIP (~100–1000 pg/mL) or GLP-1 (~10–60 pg/mL), which is generally observed in the postprandial setting among individuals with and without a history of GB.[8] Calculation of peptide dose based on lean mass which was slightly but not significantly different among the subjects resulted in indistinguishable plasma GIP levels between the GB and control groups. We cannot explain the lower trend of total GLP-1 concentrations or significantly lower concentrations of active GLP-1 in the CN subjects based on differences in lean mass as CN subjects had slightly higher lean mass. There was a correlation between the total and active GLP-1 levels within subjects and no difference in plasma DPP4 activity between groups; this raises the possibility that levels of plasma DPP4 activity may not accurately reflect metabolism of GLP-1 among subjects. Regardless, the difference in active GLP-1 concentration does not influence the interpretation of our primary outcome as if anything it would have muted the significantly greater insulin response to GLP-1 in the CN subjects.

Although the major findings presented in this paper seem to be at odds with current conceptions of β-cell regulation after bariatric surgery, the direct measures of incretin sensitivity are compatible with some of our previous observations. In a prior study, we compared islet and GI hormone responses to meal ingestion in a relatively large cohort of GB and matched control individuals.[15] Re-examination of the data in this report also suggests that the slope of insulin secretion per plasma concentration of GLP-1 are diminished in subjects with GB compared with CN. Furthermore, we used the GLP-1 receptor antagonist exendin-(9-39) to demonstrate a significantly greater effect of GLP-1 to potentiate insulin secretion in GB compared with CN subjects.[14] Blocking the GLP-1 receptor uncovered a relative ~2-fold increase in GLP-1 action at a time when GLP-1 concentrations were ~10-fold elevated. Our assumption here is that surgery leads to elevated GLP-1 secretion and β-cells respond to this. While this seems logical, it is also necessary to consider whether increased GLP-1 secretion is a response to blunted β-cell function; this hypothesis would require careful

longitudinal measures. Regardless, the presence of massive elevations of plasma GLP-1, even with blunted sensitivity to GLP-1, is likely to expose GB subjects to a substantial insulinotropic effect. This putative compensation contrasts with GB patients who have the hyperinsulinaemic hypoglycaemia syndrome as these individuals have glucose tolerance restored when given a GLP-1 receptor antagonist.[8 26] While further investigation is needed to evaluate the differences among those with and without hypoglycaemia after GB, it is plausible that the affected subjects are not able to sufficiently decrease their β-cell responses to glucose and/or GLP-1.

In our protocol, with separate infusions of GIP and GLP-1 at moderate hyperglycaemia in fasting subjects, changes in insulin secretion can be attributed to one or the other incretin alone. The attenuated responses to the incretins in the GB subjects with at least equivalent exposure to the peptides indicate a consistent, fixed change in β-cell function after surgery. While our cross-sectional design does not permit any insight into the temporal pattern of this change, it is important to note that our subjects were sufficiently distant from their operation to have had their major weight loss from GB; numerous studies demonstrate a parallel course of improved insulin sensitivity to that of body weight.[27–29] In an earlier report, we noted a comparable blunting of β-cell sensitivity to glucose after GB in a separate cohort and proposed that this was an adaptation to prevent excessive insulin secretion in response to the relative hyperglycaemia presented by their meals.[17] The present results allow this hypothetical model to be extended to include incretin-stimulated insulin secretion. Testing this model fully would require assessments similar to those described here in a longitudinal study to determine the course of β-cell function in the context of altered postprandial glucose patterns and insulin sensitivity over the time from surgery.

In subjects with diabetes, the relationship between chronic hyperglycaemia and β-cell sensitivity to incretins has been tested using infusions of GLP-1 and GIP before and after a 4-week period of intensive insulin treatment to lower glucose.[30 31] Treatment to improve glycaemic control significantly improved the β-cell response to GLP-1 and GIP in these studies. Similarly, lowering blood glucose with insulin improved the incretin effect in subjects with diabetes.[32] While underlying mechanisms for these observations are not fully understood, continuous exposure of islet cells to hyperglycaemia,[33] GLP-1[34] or GIP[35] in vitro can lead to attenuated signalling either by receptor desensitisation or reduced expression. It should be noted that chronic administration of GLP-1[36] or GLP-1r agonists[37] in individuals with T2DM does not diminish GLP-1r biological activities as glycaemic control remains steady. This link between blood glucose and insulin responses to glucose and incretins raises the possibility that the recurrent prandial hyperglycaemia or exaggerated GLP-1 secretion experienced by patients with GB could contribute to altered β-cell function.

In the group of subjects studied here, we noted a substantial effect of GB to increase insulin clearance, likely by greater hepatic extraction. This effect was present in the GIP and GLP-1 ramps as well as in the glucose clamp without incretins, indicating that it was not a function of differing insulin concentrations alone.[38 39] Increased hepatic insulin clearance after GB surgery has been reported as early as 1 week from GB[27] with similar trends observed up to 1 year compared with estimated values from before surgery. Changes in insulin clearance after GB seems to be independent of weight loss as comparable weight loss due to dietary restriction had no effect on insulin clearance estimated by insulin clamp.[40] Similarly, a cross-sectional comparison of insulin clearance between subjects several years after surgery and BMI-matched non-operated individuals showed a larger insulin clearance after GB.[41] This effect is also consistent with the β-cell changes we previously noted to glucose in a separate cohort[17] and here to incretins, in that it would tend to reduce the exposure of peripheral tissues to insulin and reduce the risk of hypoglycaemia.

There are several limitations to our study that need consideration. First, the sample size involved in this set of experiments is small and may not be reflective of the greater population of patients with GB. However, the GB group had the female predominance generally found in bariatric surgery cohorts[20] and was similar to the CN group on BMI and age. In addition, there were no substantial effects of time since surgery, absolute weight loss or lean mass on insulin secretion, suggesting a little impact of key variables that differed among the GB group. A second potential weakness is the cross-sectional design of the study. Much of the groundbreaking work in the area of bariatric surgery is based on comparisons of subjects before and after surgery[1 27 28 42 43] where changes in individuals can be tracked over time and even small differences found to be consistent. However, for outcomes where there is a very large effect size of surgery, such as we report here, the benefit of a within-subjects study design is less important. There was almost no overlap in β-cell sensitivity to incretins between these two groups of subjects, who differed primarily on the basis of having had surgery. Considering that our GB subjects had no known history of diabetes our findings cannot provide any insight into the effect of GB on β-cell function in patients with T2DM. Finally, we did not perform meal tests on our cohort of patients without GB nor we co-infused GLP-1 and GIP during hyperglycaemic clamp, therefore, we cannot compare incretin secretion and incretin sensitivity within subjects or able to comment on possible synergistic effects of the two incretins on β-cell function during clamp setting. However, it seems beyond question that the GB subjects would have vastly greater GLP-1 secretion than the controls after eating and likely GIP levels that would be comparable.[3 8 11–13 15 23]

In summary, individuals with GB for several years have reduced β-cell sensitivity to GIP and GLP-1. This blunted response to the incretins is independent of insulin sensitivity and consistent across our GB cohort. Taken together with our previous results showing decreased β-cell sensitivity to glucose in this cohort, these results support a model of attenuated insulin secretory responsiveness to the major meal-induced islet stimuli in persons adapted to GB. This model would provide an explanation for intact glucose homeostasis in GB, a setting with recurrent prandial elevations of glycaemic and incretin stimulation.

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