An obese brain and an inflamed body: Central and peripheral consequences of obesity

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Hepatic and peripheral insulin sensitivity do not improve 2 weeks after bariatric surgery


Abstract

**Background:** Bariatric surgery has rapid metabolic effects on glucose metabolism before the occurrence of clinically significant weight loss. This suggests an acute effect of the surgery itself, e.g., resulting from bypassing the nutrient flow from the proximal gastrointestinal tract. We aimed to define rapid effects of Roux-en Y gastric bypass surgery (RYGB) on glucose metabolism.

**Methods:** We studied glucose metabolism and total triglyceride hydrolysis in the basal state and during a hyperinsulinemic euglycemic clamp using stable isotopes two weeks before and two weeks after RYGB. We included 18 pre-menopausal women scheduled for RYGB. Two weeks after RYGB median weight loss was 7.8 kg.

**Results:** Basal insulin and glucose levels decreased after surgery. Endogenous glucose production (EGP) was lower after surgery. Also, insulin levels were lower during the clamp after surgery, suggesting enhanced clearance. Hepatic and peripheral insulin sensitivity did not change. Free Fatty Acid (FFA) levels increased after surgery both in the basal state and during the first step of the clamp. Total triglyceride hydrolysis did not change in the basal state and tended to be higher during hyperinsulinemia.

**Conclusions:** Within 2 weeks, RYGB reduces basal EGP as well as insulin and glucose levels without an acute beneficial effect on hepatic or peripheral insulin sensitivity. The latter may be explained by higher rates of lipolysis and exposure to FFA induced by the hypocaloric state.
Introduction

At present, bariatric surgery is the most effective treatment modality to induce sustained weight loss and reversal of the obesity-induced changes in lipid and glucose metabolism. It reduces cardiovascular risk factors and decreases mortality rates (1, 2). Bariatric procedures mainly result in reduced food intake with subsequent weight loss. Roux-en-Y gastric bypass surgery (RYGB) and biliopancreatic diversion are the most effective methods in terms of sustained control of glucose homeostasis (3). Caloric restriction and weight loss are well known mechanisms for improved insulin sensitivity (4). However, recent reports have been published on amelioration of insulin sensitivity within days after bariatric surgery (5, 6). This phenomenon occurred in the absence of significant weight loss (7-9). Traditional glucoregulatory factors, like free fatty acids (FFA) and adiponectin could not explain this early improvement. The beneficial effects on glucose metabolism were accompanied by a change in gut peptide (incretins) secretion (6, 7), i.e., an increased GLP-1 response to an oral glucose load, which could be responsible for an enhanced glucose-induced insulin response and hence lower plasma glucose. Another hypothesis proposes that by bypassing the proximal gastrointestinal tract from the nutrient flow, decreased secretion of substances with an anti-incretin effect is induced, resulting in improvement of glucose metabolism (3). However, this hypothesis has not been validated to date. In addition, it is not clear whether the improvement in glucose metabolism is due to an increase in β-cell function, an increase in hepatic or peripheral insulin sensitivity, or a combination of these factors.

In the present study we report the short-term effects on glucose metabolism and lipolysis in obese women undergoing RYBG. We performed a two-step hyperinsulinemic euglycaemic clamp with stable isotopes to determine hepatic and peripheral insulin sensitivity and lipolysis before and two weeks after surgery. We hypothesized that RYGB increases hepatic and peripheral insulin sensitivity.

Subjects and methods

Subjects

Eighteen obese women scheduled for RYGB surgery were included in this observational intervention study, and served as their own controls. These women were recruited from the outpatient clinics of the Rijnstate Hospital in Arnhem and the Slotervaart Hospital in Amsterdam, from October 2008 until December 2010. The patients were eligible for the study if they met the criteria for bariatric surgery and were scheduled to undergo RYGB surgery, if they had no DSM IV diagnosis, were older than 18 years, understood the objective of the study, and were competent to give informed consent. This competency was evaluated by the investigator and the nursing team and surgeon involved in the treatment of the patient. Exclusion criteria were: insulin dependent DM; a recent history (6 months or less) of substantial alcohol or drug abuse; the use of antipsychotic medication or antidepressant medication; any somatic illness except for obesity-related conditions (hypertension, dyslipidemia and DM
treated with oral anti-diabetics); and no informed consent. Substance (ab)use and physical health were assessed by the team involved in the pre-assessment for surgery. The study was approved by the Medical Ethical Committee of the Academic Medical Center (AMC) of the University of Amsterdam. After a complete description of the study had been given, written informed consent was obtained.

Surgical procedure

The surgical procedures were carried out in two medical centers (Rijnstate Hospital, Arnhem and Slotervaart Hospital, Amsterdam) and performed by experienced bariatric surgeons. During surgery, the gastric volume was reduced by stapling off a 30-mL proximal gastric pouch and connecting the antecolic alimentary limb in a gastroenterostomy. The biliopancreatic limb with a length of 45–50 cm from the ligament of Treitz was connected to this alimentary limb at a distance of 100–150 cm as an enteroenterostomy. This procedure resulted in a bypass of the distal stomach, duodenum, and proximal part of the jejunum.

Hyperinsulinemic euglycemic clamp

Subjects were admitted to the Metabolic Clinical Research Unit of the AMC and were studied in the supine position. After a 10-h fast from 22:00 PM the day before, a catheter was inserted into the dorsal vein of the hand or distal vein of each arm. One catheter was used for sampling of arterialized blood using a heated hand box (60°C). The other catheter was used for infusion of \([6,6-^2\text{H}_2]\text{glucose}, [1,1,2,3,3-^2\text{H}_5]\text{glycerol, glucose 20\%, and insulin. At 09:00 AM (t= -2), after drawing a blood sample for background enrichment of plasma glucose and glycerol, a primed-continuous infusion of [6,6-^2\text{H}_2]\text{glucose (99\% enrichment; Cambridge Isotopes, Andover, MA) and of [1,1,2,3,3-^2\text{H}_5]\text{glycerol (99\% enrichment; Cambridge Isotopes, Andover, MA) were started at a rate of 0.11 \mu\text{mol/kg/min after a priming dose equivalent to 120 min infusion. After 110, 115 and 120 min, blood samples were drawn for determination of glucose and glycerol enrichments, glucoregulatory hormones and FFA. Subsequently, at 11:05 AM (t=0), a continuous infusion of insulin (Actrapid 100U/ml; Novo Nordisk Farma, Alphen a/d Rijn, the Netherlands) was started for 2h at a rate of 20 \text{mU/m}^2\text{ body surface area min}^{-1}. At t=2, the infusion rate of insulin was increased to 60\text{mU/m}^2\text{ body surface area. Plasma glucose was measured every 10 min and glucose 20\% was infused at a variable rate to maintain plasma glucose at 5.0 mmol/liter. [6,6-^2\text{H}_2]\text{glucose was added to the 20\% glucose solution to achieve glucose enrichments of 1\% to minimize changes in isotopic enrichment due to changes in the infusion rate of exogenous glucose. At t = 2 and t = 4, 5h blood samples with a 5 min interval were drawn to measure glucose and glycerol enrichments and 2 samples were drawn to measure glucoregulatory hormones and FFA. During the study the participants were only allowed to drink water.}}\]
Body composition and indirect calorimetry

Body composition was measured using bioelectrical impedance analysis (Maltron BF-906, Rayleigh, UK). Oxygen consumption (VO₂) and CO₂ production (VCO₂) were measured continuously during the final 20 min of the basal state and the hyperinsulinemic euglycemic clamp by indirect calorimetry using a ventilated hood system (Sensormedics model 2900; Sensormedics, Anaheim, USA) and the final 10 min were used for calculations of the respiratory exchange ratio (RER).

Glucose and lipid metabolism measurements

Plasma glucose concentrations were measured with the glucose oxidase method using a Biosen C-line plus glucose analyzer (EKF Diagnostics, Barbleben/Magdeburg, Germany). Plasma FFA concentrations were determined with enzymatic colorimetric method (NEFA-C test kit; Wako Chemicals, Neuss, Germany) with an intra-assay variation of 1%, inter-assay variation of 4-15% and a detection limit of 0.02 mmol/L. [6,6-2H₂]glucose and [1,1,2,3,3-2H₅]glycerol enrichment (tracer-to-tracee ratio) were measured as described earlier (8, 9).

Insulin and cortisol were determined on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA, USA). Insulin was measured with a chemiluminescent immunometric assay with intra-assay variation of 4–5%, inter-assay variation of 5% and detection limit of 15 pmol/l. Cortisol was measured with a chemiluminescent immunoassay with intra-assay variation of 3-6%, inter-assay variation of 5-7% and a detection limit of 50 nmol/l. Glucagon was determined with the Linco 125I RIA (Linco Research, St Charles, MO, USA) with an intra-assay variation of 4-8%, inter-assay variation of 6-11% and detection limit of 15 ng/l. C-peptide was determined with a 125I radioimmunoassay (Linco Research, Inc, USA). Intra-assay variation 4-8%, inter-assay variation 9-16%, detection limit 50 pmol/L.

Calculations and statistical analyses

Each subject served as its own control. Data were analyzed using non-parametric tests. Comparison of the data before surgery compared to the data obtained two weeks after surgery were analyzed using the wilcoxon signed rank test. SPSS version 16.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Data are presented as median and interquartile range. Comparisons were considered statistically significant if the P value was <0.05.

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula described previously by Matthews et al. (10) Endogenous glucose production (EGP) and peripheral glucose uptake (rate of disappearance [Rd]) were calculated using the modified form of the Steel equation as described previously (11, 12). EGP is expressed as μmol(kg FFM)⁻¹min⁻¹ (FFM, fat-free mass) and Rd as μmol(kg⁻¹min⁻¹). Insulin clearance was calculated as the rate of insulin infusion (mU [m² body surface area] min⁻¹) divided by the mean plasma insulin concentration during the clamp (13).
Resting energy expenditure (REE), glucose oxidation and fat oxidation rates were calculated from VO$_2$ and VCO$_2$ as reported previously (14). Total triglyceride hydrolysis is expressed per REE (μmol/kcal) as suggested by Koutsari et al. (15).

**Results**

**Study participants.**

We included 18 pre-menopausal Caucasian women (median age 40.5 [26-50] yrs, median BMI 42.9 [38.7-61.3] kg/m2) scheduled for RYGB. None of the subjects had type 2 diabetes. Two weeks after RYGB, median weight loss was 7.8kg [2-14 kg], which corresponds to 6.2% (2 – 14 %) weight loss.

**Glucose metabolism.**

The second step of the hyperinsulinemic euglycemic clamp was unsuccessful in one subject before surgery, and in two subjects after surgery. In addition, the first and second step of the hyperinsulinemic clamp were unsuccessful in one subject after surgery, in all cases due to technical failures with the iv-lines. Therefore the paired results shown in the tables represent 18 women in the basal state before and after surgery and 15 women in the hyperinsulinemic state before and after surgery.

HOMA-IR decreased significantly after surgery suggesting enhanced insulin sensitivity (table 1). Basal glucose, insulin and C-peptide levels decreased after surgery. Plasma cortisol was similar, while glugagon increased after surgery (table 1).

Endogenous glucose production (EGP) decreased significantly 2 weeks after the RYGB. Hepatic insulin sensitivity expressed as percentage suppression of EGP by insulin was assessed during the first step of the hyperinsulinemic clamp (insulin before surgery 263 [145-450] pmol/L vs after surgery 189 [130-278] pmol/L, p < 0.001) and showed no significant difference between the preoperative and postoperative condition. In addition, the correlation coefficient between EGP and circulating insulin levels did not differ before versus after surgery (data not shown).

Insulin levels during the second step of the hyperinsulinemic euglycemic clamp were significantly lower after surgery (table 1). Therefore we corrected Rd for circulating insulin levels (Rd/[insulin]). Insulin-mediated peripheral glucose uptake (Rd) showed no significant difference between the preoperative and postoperative condition. (fig 2).

**Lipid metabolism and REE**

REE was significantly lower in the basal state after surgery (table 2). The increase in REE during the hyperinsulinemic clamp was blunted, resulting in lower REE during hyperinsulinemia after surgery. FFA in the basal state increased after surgery and remained higher during the first step of the clamp.

Total triglyceride hydrolysis expressed per REE remained stable in the basal state and tended to be higher during the first step of the clamp after surgery.
HEPATIC AND PERIPHERAL INSULIN SENSITIVITY DO NOT IMPROVE 2 WEEKS AFTER BARIATRIC SURGERY

Table 1. Glucose and lipid metabolism in the basal state and during the hyperinsulinemic euglycemic clamp.

<table>
<thead>
<tr>
<th></th>
<th>BASAL STATE (N = 18)</th>
<th>HYPERINSULINEMIC CLAMP (N = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before surgery</td>
<td>After surgery</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.3 (1.6 – 3.9)</td>
<td>1.5 (0.9 – 2.4)</td>
</tr>
<tr>
<td>GLUCOSE (MMOL/L)</td>
<td>5.4 (4.8 – 6.3)</td>
<td>4.8 (4.6 – 5.3)</td>
</tr>
<tr>
<td>EGP (µMOL/KG FFM.MIN-1)</td>
<td>13 (12.7 – 14.5)</td>
<td>11.4 (10.5 – 12.9)</td>
</tr>
<tr>
<td>SUPRESSION OF EGP (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RD ([µMOL.L/KG.MIN].[PMOL]*10-2]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>INSULIN (PMOL/LL)</td>
<td>81 (62.3 – 111)</td>
<td>48 (30 – 67)</td>
</tr>
<tr>
<td>C-PEPTIDE (PMOL/LL)</td>
<td>950 (757 – 1127)</td>
<td>805 (540 – 596)</td>
</tr>
<tr>
<td>GLUCAGON (NG/LITER)</td>
<td>51 (39 – 65)</td>
<td>69 (53 – 78)</td>
</tr>
<tr>
<td>CORTISOL (NMOL/LITER)</td>
<td>250 (182 – 365)</td>
<td>199 (166 – 277)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range). Endogenous glucose production (EGP) was assessed during the first step of the hyperinsulinemic clamp. It was completely suppressed during the second step of the clamp.

Table 2. Lipid metabolism measurements in the basal state and during the hyperinsulinemic euglycemic clamp.

<table>
<thead>
<tr>
<th></th>
<th>BASAL STATE (N = 18)</th>
<th>HYPERINSULINEMIC CLAMP (N = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before surgery</td>
<td>After surgery</td>
</tr>
<tr>
<td>FFA (MMOL/L)</td>
<td>0.78 (0.72 – 0.84)</td>
<td>0.94 (0.87 – 1.09)</td>
</tr>
<tr>
<td>TTGH (µMOL/KCAL)</td>
<td>291 (243 – 354)</td>
<td>276 (235 – 330)</td>
</tr>
<tr>
<td>REE (KCAL/DAY)</td>
<td>1858 (1682 – 2017)</td>
<td>1731 (1480 – 1915)</td>
</tr>
<tr>
<td>GLUCOSE OXIDATION (µMOL/KG.MIN)</td>
<td>1.8 (0.25 – 3.1)</td>
<td>0.45 (0 – 1.74)</td>
</tr>
<tr>
<td>FAT OXIDATION (µMOL/KG.MIN)</td>
<td>1.1 (0.9 – 1.2)</td>
<td>0.6 (0.26 – 0.8)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range). TTGH= total triglyceride hydrolysis.
Discussion

We studied the short-term metabolic effects of Roux-en-Y gastric bypass surgery to explore whether the assumed increase in insulin sensitivity could be explained by an increase in hepatic or peripheral insulin sensitivity. We found that within two weeks, bariatric surgery reduces basal EGP, insulin and glucose levels without a beneficial effect on hepatic or peripheral insulin sensitivity. Our results are in line with previous studies (17, 18), which showed no increase in peripheral insulin sensitivity. However, hepatic insulin sensitivity was not assessed in those studies. Surprisingly, we did observe a significant decrease in HOMA-IR and also found that the range in HOMA-IR was smaller after surgery, indicating that the reduction in basal insulin and glucose levels in hypocaloric conditions might be part of a preserved metabolic adaptation in all subjects. Also, HOMA-IR at baseline did not correlate with clamp-derived Rd-rates (data not shown), suggesting that measuring HOMA-IR in morbidly obese women before and after surgery does not reflect true insulin sensitivity. Lower HOMA-IR after surgery might be explained by lower EGP and lower insulin secretion or enhanced insulin clearance rates. The latter is in line with the lower insulin levels during insulin infusion after surgery in our subjects. Although clamp-derived insulin sensitivity was not changed in the short term, earlier studies have shown improvements in glucose tolerance using an oral glucose tolerance tests (19). An enhanced β-cell responsivity or incretin response to an oral glucose load might explain this difference. However, the altered anatomy of the gastrointestinal tract might interfere with glucose absorption and hence lower glucose concentrations after an oral glucose load without frankly changing the β-cell response per se.
Besides lower glucose levels and lower EGP, higher levels of FFA all indicate that our subjects were in a state of prolonged fasting which is known to ameliorate glucose metabolism even in patients with DM2 (20). Also, caloric restriction is known to reduce the incidence of diabetes (21). A very low calorie diet in obese patients with DM2 has been shown to have no effect on hepatic insulin sensitivity in the short term while Markovic et al. (22) did report an increase in hepatic insulin sensitivity 4 days after a hypocaloric diet in obese subjects. These contradictory results might be explained by either a difference in study population or a difference in composition of the hypocaloric diet. Our subjects were mainly on a liquid or pureed diet. Our findings suggest that the major short-term effect of bariatric surgery is inducing a state of prolonged fasting with beneficial effects on basal endogenous glucose production and hence glucose levels.

Higher FFA levels during hyperinsulinemia in our subjects might explain why peripheral and hepatic insulin sensitivity did not change in the short term. FFAs are known to interfere with insulin signaling (23). The increase in FFA in the first weeks after RYGB has been described previously (24) and FFA levels returned to pre-surgery levels after one year, a time span which has been shown to be sufficient to reduce the incidence of diabetes in patients after bariatric surgery (1). Total triglyceride hydrolysis measured with labeled glycerol and expressed in relation to REE tended to be higher during hyperinsulinemia only. The difference between the higher basal FFA concentrations and stable basal tracer-derived lipolytic flux can be explained by either higher incomplete lipolysis (25) or reduced FFA uptake.

REE was lower after surgery and did not increase during hyperinsulinemia. Lowering REE is a general metabolic adaptation to a hypocaloric state (26). The blunted insulin/glucose-mediated increase in REE suggests either a different thermic effect of glucose or different metabolic handling of infused glucose. This warrants further research.

In conclusion, the beneficial early metabolic effects described in morbidly obese adults undergoing bariatric surgery are not caused by an increase in insulin sensitivity. A state of prolonged fasting induced by the RYGB explains lower endogenous glucose production rates with subsequent lower plasma glucose levels. Lower insulin levels in the basal state and during the hyperinsulinemic clamp indicate enhanced hepatic insulin clearance. Therefore lower HOMA-IR in this population does not truly reflect insulin sensitivity. The lack of effect on insulin sensitivity might be explained in part by higher levels of free fatty acids.

Reference list

CHAPTER 5


