Diabetologia (2011) 54:2093–2102
DOI 10.1007/s00125-011-2193-6

ARTICLE

Early and longer term effects of gastric bypass surgery on tissue-specific insulin sensitivity and beta cell function in morbidly obese patients with and without type 2 diabetes

S. Camastra · A. Gastaldelli · A. Mari · S. Bonuccelli · G. Scartabelli · S. Frascerra · S. Baldi · M. Nannipieri · E. Rebelos · M. Anselmino · E. Muscelli · E. Ferrannini

Received: 23 February 2011 / Accepted: 22 April 2011 / Published online: 26 May 2011

Abstract
Aims/hypothesis Bariatric surgery consistently induces remission of type 2 diabetes. We tested whether there are diabetes-specific mechanisms in addition to weight loss.
Methods We studied 25 morbidly obese patients (BMI 51.7±1.5 kg/m^2 [mean±SEM]), 13 with non-insulin-treated type 2 diabetes (HbA_{1c} 7.1±0.5% [54±5 mmol/mol]), before and at 2 weeks and 1 year after Roux-en-Y gastric bypass (RYGB). Lean (n=8, BMI 23.0±0.5 kg/m^2) and obese (n=14) volunteers who were BMI-matched (36.0±1.2) to RYGB patients at 1 year after surgery served as controls. We measured insulin-stimulated glucose disposal (M) and substrate utilisation (euglycaemic clamp/indirect calorimetry), endogenous glucose production (EGP) by 6,6-[$^2H_2$]glucose, lipolysis (rate of appearance of [$^2H_5$]glycerol) and beta cell function (acute insulin response to i.v. glucose [AIR] as determined by C-peptide deconvolution).
Results At baseline, all obese groups showed typical metabolic abnormalities, with M, glucose oxidation and non-oxidative disposal impaired, and EGP, lipolysis, lipid oxidation and energy expenditure increased. Early after RYGB plasma glucose and insulin levels, and energy expenditure had decreased, while lipid oxidation increased, with M, EGP and AIR unchanged. At 1 year post-RYGB (BMI 34.4±1.1 kg/m^2), all diabetic patients were off glucose-lowering treatment and mean HbA_{1c} was 5.4±0.14% (36±2 mmol/mol) (p=0.03 vs baseline); AIR also improved significantly. In all RYGB patients, M, substrate oxidation, EGP, energy expenditure and lipolysis improved in proportion to weight loss, and were therefore similar to values in obese controls, but still different from those in lean controls.
Conclusions/interpretation In morbidly obese patients, RYGB has metabolic effects on liver, adipose tissue, muscle insulin sensitivity and pattern of substrate utilisation; these effects can be explained by energy intake restriction and weight loss, the former prevailing early after surgery, the latter being dominant in the longer term.

Keywords Bariatric surgery · Diabetes remission · Insulin resistance · Lipolysis · Morbid obesity · Substrate oxidation

Electronic supplementary material The online version of this article (doi:10.1007/s00125-011-2193-6) contains supplementary material, which is available to authorised users.

S. Camastra (✉) · S. Bonuccelli · S. Frascerra · S. Baldi · M. Nannipieri · E. Rebelos · E. Muscelli · E. Ferrannini
Department of Internal Medicine, University of Pisa,
Via Roma, 67,
56100 Pisa, Italy
e-mail: camastra@ifc.cnr.it

A. Gastaldelli · E. Ferrannini
Institute of Clinical Physiology, C.N.R.,
Pisa, Italy

A. Mari
Institute of Biomedical Engineering, C.N.R.,
Padua, Italy

G. Scartabelli
Department of Endocrinology, University of Pisa,
Pisa, Italy

M. Anselmino
Division of Bariatric Surgery, Santa Chiara Hospital,
Pisa, Italy

Abbreviations

AIR	Acute insulin response to i.v. glucose
EGP	Endogenous glucose production
FFM	Fat-free mass
GLP-1	Glucagon-like peptide-1
IQR	Interquartile range
M	Insulin-stimulated glucose disposal
M_{EE}	M by basal energy expenditure
M_{FFM}	M per kg of FFM
R_a	Rate of appearance
R_aGly	Glycerol rate of appearance
RYGB	Roux-en-Y gastric bypass
TGD	Total glucose disposal

Introduction

Bariatric surgery improves multiple metabolic abnormalities in a large proportion of morbidly obese patients [1]. In severely obese patients with type 2 diabetes, bariatric operations consistently improve or reverse type 2 diabetes [1]. Although few studies have employed techniques such as the euglycaemic–hyperinsulinaemic clamp, which directly measures in vivo insulin sensitivity [2–4], improved insulin resistance is common in bariatric patients post-surgery, as reviewed by Ferrannini and Mingrone [5]. In general, insulin resistance has improved roughly in proportion to weight loss and is therefore imputable to it [4, 5]. However, some studies, as reviewed by Rubino et al. [6], have suggested that with surgery, insulin sensitivity increases to a greater degree than that explained by the amount of weight lost, or it increases before significant weight loss occurs. Furthermore, insulin secretion has been found to be increased following surgery-induced weight loss, despite the abatement of insulin resistance [5, 7]. These observations have led to the hypothesis that bariatric surgery or at least some kinds of bariatric procedures may have effects on glucose metabolism that are independent of weight loss, i.e. diabetes-specific [8]. This speculation has received support by the mounting evidence of favourable changes in gastrointestinal hormones following surgery. For example, in patients undergoing Roux-en-Y gastric bypass (RYGB), the most common type of bariatric operation worldwide, serum levels of glucagon-like peptide-1 (GLP-1) increase early after surgery, thereby potentiating glucose-induced insulin secretion [9]. However, to the best of available evidence, GLP-1 does not affect peripheral insulin sensitivity [10]. Ghrelin, on the other hand, has been found to be reduced after RYGB in some but not all studies, according to a review by Field et al. [11], although, when exogenously infused, it can depress insulin sensitivity and beta cell function [12]. The predicament of diabetes-specific surgery is further complicated by the fact that bariatric surgery encompasses several different procedures, ranging from those that are purely restrictive, such as gastric banding, through to those that have a component of malabsorption (e.g. RYGB) and those with predominantly malabsorptive components (e.g. biliopancreatic diversion), all of which are often executed in variants [13]. Moreover, it is not clear whether the response to surgery in patients with diabetes may involve different mechanisms from those in non-diabetic individuals [14].

All in all, the notion that anatomical alterations of the bowel and changes in food transit or contact with the gastrointestinal mucosa may influence glucose homeostasis through hormonal, metabolic or neural signals is of high physiological interest and may provide new insight into the pathogenesis of type 2 diabetes. We therefore tested this hypothesis systematically, by assessing insulin sensitivity at the level of the liver, adipose tissue and muscle (using the clamp technique combined with tracer infusion), and relating them to changes in body composition, energy expenditure and substrate utilisation pattern. We obtained measurements early (2 weeks) and at 1 year following laparoscopic RYGB in non-diabetic individuals, patients with type 2 diabetes and in appropriately matched control participants.

Methods

Participants The study group included 13 morbidly obese patients with type 2 diabetes and 12 age-, sex- and BMI-matched non-diabetic patients undergoing laparoscopic RYGB. Diabetes was newly diagnosed in four patients, while in the other nine patients known diabetes duration was 3.2±1.1 years (range 1–10). Mean HbA_{1c} was 7.1± 0.5% (54±5 mmol/mol) in the whole group of type 2 diabetes patients, six of whom were being treated by diet alone and seven by oral hypoglycaemic agents (metformin four, metformin plus a sulfonylurea two, pioglitazone one). Diabetic and non-diabetic patients were studied at baseline (pre-surgery) and 1 year later (post-surgery); 11 diabetic and eight non-diabetic patients were also studied between 15 and 20 days after surgery. Two control groups were included, consisting of eight lean control and 14 obese non-diabetic volunteers whose BMI was matched to that of the RYGB patients at 1 year post-surgery (Table 1). Thus, a total of 91 metabolic studies was performed.

The study was approved by the local ethics committee. The nature and purpose of the study were carefully explained to all participants before they provided written consent to participate.

Study design At the time of study and prior to surgery, all patients were weight-stable and ambulatory. To avoid con-

Table 1 Anthropometric and metabolic characteristics

Characteristic	Controls		RYGB patients	
	Lean	Obese	Non-diabetic	Diabetic
n (all)	8	14	12	13
Men (*n*)	0	2	1	4
Women (*n*)	8	12	11	9
Age (years)	39±4	39±2	43±2	48±2*†
Body weight (kg)	63±2	98±4†	147±5*†	134±7*†
BMI (kg/m^2)	23.0±0.5	36.0±1.2†	53.7±1.8*†	49.8±2.3*†
Total body water (kg)	32.0±0.7	40.6±1.5†	52.1±2.4*†	52.4±3.9*†
FFM (kg)	44.1±0.6	54.9±2.2†	69.3±3.4*†	71.5±5.3*†
Fat mass (%)	29.4±1.5	43.8±1.8†	53.2±1.7*†	46.5±2.3†
Fasting glucose (mmol/l)	5.04±0.13	5.14±0.10	5.66±0.16	8.64±0.79*†
Fasting insulin (pmol/l)	41 (20)	86 (55)†	130 (68)*†	154 (145)*†
Steady-state insulin (pmol/l)	592 (116)	811 (284)†	759 (245)†	762 (249)†
Fasting NEFA (μmol/l)	392±53	601±53†	603±48†	689±43†
Steady-state NEFA (μmol/l)	6±3	50±7†	68±12†	136±27†
Fasting glycerol (μmol/l)	59±6	102±11†	118±9†	121±7†
Steady-state glycerol (μmol/l)	29±2	48±6†	72±7*†	81±12*†
M (μmol/min)	2,773 (902)	1,831 (772)†	1,653 (953)†	1,179 (1,176)*†
M_{EE} (μmol/kJ)	722 (353)	333 (158)†	293 (138)†	176 (243)
M_{FFM} (μmol min^{-1} [kg FFM]$^{-1}$)	64.0 (17.1)	29.7 (18.0)†	26.2 (13.5)†	15.7 (22.4)*†
M_{FFM}/I (nmol min^{-1} [kg FFM]$^{-1}$ [pmol/l]$^{-1}$)	112 (46)	40 (40)†	31 (25)†	23 (25)*†
TGD_{FFM} (μmol min^{-1} [kg FFM]$^{-1}$)	65.5 (19.8)	28.9 (19.6)†	26.0 (16.9)†	15.7 (20.0)*†
EGP (μmol/min)	637 (172)	719 (148)†	859 (274)*†	844 (435)†
EGP_{EE} (μmol/kJ)	168 (42)	146 (14)	150 (34)	138 (37)
R_aGly (μmol/min)	113 (86)	228 (228)†	351 (114)†	349 (120)*†
R_aGly_{FM} (μmol min^{-1} [kg FM]$^{-1}$)	7.43 (4.67)	6.03 (2.45)	4.24 (0.84)*	6.36 (1.42)
R_aGly clamp (μmol/min)	47 (8)	165 (90)†	154 (98)†	194 (134)†
R_aGly suppression (%)	62 (24)	50 (15)	54 (9)	39 (26)†

Values are mean±SEM or median (IQR)

*$p \leq 0.05$ vs obese controls and †$p \leq 0.05$ vs lean controls, by Mann–Whitney *U* test

founding, glucose-lowering therapy was withheld 1 week before the metabolic study in the seven type 2 diabetic patients on medication. Each metabolic study consisted of a euglycaemic–hyperinsulinaemic clamp combined with infusion of tracer glucose and tracer glycerol (to measure insulin sensitivity, endogenous glucose production [EGP] [15] and lipolysis [16], respectively), and with indirect calorimetry (to measure energy expenditure and substrate oxidation rates [17]). In brief, after an overnight (12 h) fast, baseline blood samples were drawn and primed-continuous infusions of 6,6-[$^2H_2$]glucose (0.22 μmol min^{-1} kg^{-1}; prime: 22.0 μmol/kg× [FPG/5]) and ^{2}[H_5]glycerol (0.01 mg min^{-1} kg^{-1}; prime: 1.0 mg/kg) were started at −180 min via an antecubital vein catheter. At time −40, −20, −10 and 0 min blood samples were obtained from an arterialised vein for measurement of glucose, NEFA and insulin, and for determination of tracer steady state. At time zero, a primed-continuous insulin (Humulin R; Eli Lilly, Indianapolis, IN, USA) infusion (at a rate of 240 pmol min^{-1} m^{-2}) was started and continued for 180 min in type 2 diabetic patients (120 min in non-diabetic patients). At time 0, the 6,6-[$^2H_2$]glucose infusion was decreased (to 0.11 μmol min^{-1} kg^{-1}) and continued until the end of the clamp. During the clamp, a variable rate of a 20% (wt/vol.) glucose solution, enriched with 6,6-[$^2H_2$] glucose, was infused to maintain plasma glucose concentration (5.5 mmol/l) and enrichment constant. Plasma glucose levels were measured every 5 min throughout the clamp. Plasma insulin, glycerol and NEFA concentrations, and 6,6-[$^2H_2$]glucose and [$^2H_5$]glycerol enrichment were measured every 20 min after the start of insulin. In diabetic patients only, a glucose bolus (11.5 g/m body height glucose as a 50% (wt/vol.) aqueous solution) was injected into a

peripheral vein at the end of the clamp and blood samples taken every 2 min for the next 8 min for measurement of plasma glucose and C-peptide concentrations [18].

Indirect calorimetry was used to estimate energy expenditure and substrate oxidation rates. During the basal period (from −40 to 0 min) and the steady-state period of the clamp (last 40 min), indirect calorimetry was performed using a computerised, continuous, open-circuit system with a canopy (Vmax 29 N; SensorMedics,Yorba Linda, CA, USA). Body composition was evaluated by electrical bioimpedance (TBF 300; Tanita, Tokyo, Japan) with standard formulas [19, 20].

Surgical procedure In participants undergoing laparoscopic RYGB, a small proximal gastric pouch of 30 ml was created with several firings of a linear stapling endocutter; the jejunum was divided 120 cm distal to the ligament of Treitz and a 2 cm end-to-side gastrojejunostomy was performed using a hand-sewn technique. A side-to-side jejunojejunostomy was then created 150 cm distal to the gastrojejunostomy [21].

Analytical procedures Plasma glucose was measured by the glucose oxidase technique on a glucose analyser (Beckman, Fullerton, CA, USA). Plasma insulin and C-peptide were assayed by a specific radioimmunoassay (Linco Research, St Charles, MO, USA; and MYRIA; Technogenetics, Milan, Italy, respectively). Plasma NEFA and glycerol concentrations were measured spectrophotometrically by an automated procedure (Synchro CX4; Beckman, Brea, CA, USA).

Data analysis In the basal state, EGP was calculated as the glucose rate of appearance (R_a) using the steady-state equation for stable isotopic tracers; during the clamp, glucose R_a was calculated using a two-compartment model, as previously described [15]. Insulin-stimulated glucose disposal (M) in μmol/min was calculated as the mean exogenous glucose infusion rate during the last 40 min of the clamp corrected for changes in glucose concentration within a distribution volume of 200 ml per kg body weight. M values were also expressed per kg of fat-free mass (FFM) (M_{FFM}, in μmol min^{-1} [kg FFM]$^{-1}$). Total glucose disposal (TGD) was calculated from the tracer data, averaged over the last 40 min of the clamp and expressed in the same units as M_{FFM} (TGD_{FFM}, in μmol min^{-1} [kg FFM]$^{-1}$). To correct for differences in steady-state plasma insulin concentrations during the clamp, we also calculated an index of peripheral insulin sensitivity (M_{FFM}/I, in μmol min^{-1} [kg FFM]$^{-1}$ [pmol/l]$^{-1}$) as the ratio of M_{FFM} to the steady-state plasma insulin concentrations [22]. Acutely after surgery bioimpedance measurements of FFM are biased by dehydration [23]; for this reason M values were also corrected for the basal rate of energy expenditure, which represents the total mass of metabolically active tissues [22], an approach previously used in humans [24].

Lipolysis was estimated as the glycerol rate of appearance (R_aGly) in μmol/min, and calculated using steady-state equations for the fasting state and Steele's equations during the clamp, as previously described [16]. R_aGly was also expressed per kg of fat mass (R_aGly_{FM}, in μmol min^{-1}[kg FM]$^{-1}$).

With regard to beta cell function, fasting insulin secretion rate was calculated by deconvolution of fasting C-peptide concentrations and expressed in pmol min^{-1} m^{-2}. The acute insulin response to i.v. glucose (AIR) in pmol min^{-1} m^{-2} $(mmol/l)^{-1}$ was calculated as the ratio of incremental insulin secretion to incremental glucose concentrations during the 8 min following glucose injection; investigators from our team have previously provided evidence that AIR values calculated from a glucose bolus injected at the end of a euglycaemic clamp are similar to those obtained when the bolus is administered in the fasting state [18].

Energy expenditure and net substrate oxidation rates were calculated from gas exchange measurements as previously described [25]. Non-oxidative glucose disposal (reflecting, for the most part, glycogen synthesis) was obtained as the difference between total and oxidative glucose disposal. Lipid oxidation, and oxidative and non-oxidative glucose disposal rates were normalised by the basal rate of energy expenditure.

Statistical analysis Data are given as mean±SEM or, if non-normally distributed, as median (interquartile range [IQR]). Kruskal–Wallis and Mann–Whitney U tests were used to compare group values, whereas individual group differences were analysed by the Bonferroni–Dunn test. Treatment-induced changes were tested by Wilcoxon's signed rank test. Simple associations were tested by calculating Spearman's rank correlation coefficient (ρ), data fitting was performed by non-linear regression techniques. Statistical analyses were carried out using JMP 3.1 software (SAS Institute, Cary, NC, USA); a value $p \leq 0.05$ was considered significant.

Results

Baseline All measures of body size, including total body water, fat and FFM, and waist circumference, were higher in the obese groups, especially in the surgical patients, than in the lean controls (Table 1). Fasting plasma glucose levels were only raised in the type 2 diabetic group (electronic supplementary material [ESM] Fig. 1b), whereas fasting plasma insulin concentrations were progressively higher across obese groups. During the clamp, steady-state plasma insulin concentrations were also higher in the obese groups as a

result of the higher total exogenous insulin infusion rate used in these participants. Basal and steady-state plasma NEFA and glycerol levels were all elevated in the obese groups.

Glucose disposal was reduced in the obese groups, whether expressed as the total quantity (M) or normalised by kg of FFM (M_{FFM}) or by basal energy expenditure (M_{EE}) or as the tracer-derived TGD (TGD_{FFM}); the values in type 2 diabetic patients were significantly (p=0.002) lower than in non-diabetic surgical participants. Fasting total EGP was higher in the obese groups than in lean controls, as was total R_aGly in the fasting state and during the clamp (Table 1), with no differences between type 2 diabetic and non-diabetic surgical patients. During the clamp, EGP was fully suppressed in all groups, whereas per cent-wise suppression of lipolysis was impaired in the type 2 diabetic group (Table 1 and ESM Fig. 2).

Basal energy expenditure was significantly increased in all obese groups (p<0.0001), the more so in RYGB patients (p<0.004). In the whole baseline dataset, basal energy expenditure was strongly correlated with lean body mass (ρ=0.88, p<0.0001) and BMI (ρ=0.78, p<0.0001). Fasting carbohydrate oxidation was lower (p=0.04) and lipid oxidation was higher (p=0.03) in obese participants than in lean controls (ESM Fig. 3a–c). During the clamp, glucose-induced thermogenesis, which averaged 7.2±1.9% (mean±SEM) in lean controls, was significantly reduced in obese (p<0.003) participants, especially those undergoing RYGB. During the clamp, glucose oxidation was stimulated and lipid oxidation was suppressed in all participants (p<0.0001 for both). However, insulin-stimulated oxidative (p=0.01) and non-oxidative glucose disposal (p=0.0003) were depressed in the obese groups, whereas lipid oxidation was higher (p<0.03) than in lean controls (Fig. 1a–c).

Early after surgery Body weight and BMI were slightly, albeit significantly, reduced in the non-diabetic and type 2 diabetic groups (ESM Fig. 1a). However, most of this change was accounted for by a reduction in total body water (Table 2), presumably reflecting postsurgical dehydration. Under these circumstances, calculation of FFM from body water leads to a significant underestimate. Fasting plasma glucose and insulin concentrations dropped more in type 2 diabetic than non-diabetic patients (Table 2, ESM Fig. 1b, c); fasting glucose was <7 mmol/l in six of 11 patients. In contrast, fasting and steady-state plasma NEFA and glycerol levels were consistently higher than at baseline. M, EGP and R_aGly were not significantly changed at this time in the obese group, while M tended to be higher and EGP tended to be lower (p=0.13) in the diabetic group (Table 3). Basal energy expenditure was reduced, fasting glucose oxidation was lower and fasting lipid oxidation was higher (p≤0.05 for all compared with controls), without differences between type 2 diabetic and non-diabetic participants (ESM Fig. 3a–c). During the clamp, EGP was suppressed, glucose-induced thermogenesis was unchanged, glucose oxidation was reduced, and lipid oxidation and non-oxidative glucose disposal were increased (p≤0.05 for all compared with controls), again to similar degrees in type 2 diabetic and non-diabetic participants (Fig. 1a–c). In parallel with enhanced lipid oxidation, insulin-induced suppression of lipolysis was lower than in lean controls (significantly so in the type 2 diabetic group) (Table 3).

At 1 year after surgery Both surgical groups had lost an average 30% of their initial body weight, which was now similar to that of the obese controls; in both groups, roughly two thirds of this loss was due to loss of fat, the remainder to loss of fat-free tissues (Table 2). Fasting glucose was further decreased in both groups (ESM Fig. 1b) and no longer different from values in lean controls (Table 2); plasma insulin dropped further (ESM Fig. 1c), but was still slightly

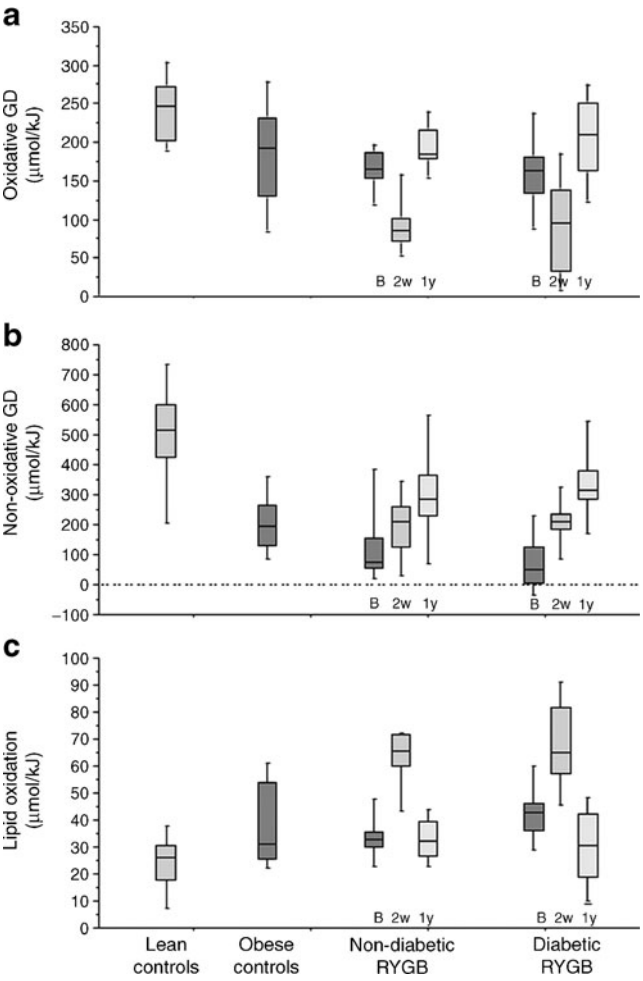

Fig. 1 Oxidative (**a**) and non-oxidative (**b**) glucose disposal (GD), and lipid oxidation (**c**) during the euglycaemic clamp in lean and obese controls, and in RYGB patients as labelled before (B), 2 weeks after (2w) and 1 year after (1y) RYGB. Data are boxplots

Table 2 Clinical and metabolic variables before and after RYGB

Variable	Non-diabetic patients					Diabetic patients				
	Pre-surgery	*p* value[a]	Early post	*p* value	1 year	Pre-surgery	*p* value	Early post	*p* value	1 year
n	12		8		12	13		11		13
Time post surgery (months)	–	–	0.5±0.02	–	13.6±1.5	–	–	0.6 ±0.03	–	13.3±0.9
Body weight (kg)	(152±6)[b]	0.01	146±6*†	0.002	101±5†	(133±9)[c]	0.003	123±8*†	0.002	88±5†
Weight change (kg)	–	–	−6.1±1.3	–	−46.0±4.8	–	–	−9.3±1.0	–	−46.2±3.0
Weight change (%)	–	–	−3.9±0.8	–	−31.1±2.7	–	–	−7.0±0.5	–	−34.5±1.0
BMI (kg/m^2)	(54.7±2.1)[b]	0.01	52.5±1.9*†	0.002	36.8±1.7†	(49.5±2.2)[c]	0.003	46.3±2.1*†	0.002	32.6±1.5
Total body water (kg)	(55.2±2.6)[b]	0.03	47.9±1.9*†	0.002	43.3±2.2†	(50.5±4.6)[c]	0.003	44.4±2.8†	0.002	42.8±2.3†
FFM (kg)	69.3±3.4	–	–	0.006	59.1±3.30†	71.5±5.3	–	–	0.002	58.5±3.2†
FM (kg)	78.1±4.2	–	–	0.002	42.1±3.4†	62.3±5.0	–	–	0.002	29.2±3.2*†
FM (%)	53.2±1.7	–	–	0.002	41.2±1.9†	46.5±2.3	–	–	0.002	32.6±2.5*
Fasting glucose (mmol/l)	5.66±0.16	0.02	5.27±0.11	0.002	4.92±0.07	8.64±0.79	0.01	6.81±0.37*†	0.002	5.22±0.21
Fasting insulin (pmol/l)	130 (68)	0.02	100 (52)†	0.002	44 (23)*	154 (145)	0.003	104 (49)†	0.002	46 (23)*
Steady-state insulin (pmol/l)	759 (245)	NS	689 (151)	0.002	522 (107)	762 (249)	NS	664 (133)*	0.004	491 (147)*
Fasting NEFA (μmol/l)	603±48	0.01	986±89*†	NS	541±39†	689±43	0.03	867±42*†	NS	672±28
Steady-state NEFA (μmol/l)	68±12	0.01	218±35*†	NS	54±24†	136±27	NS	198±55*†	0.005	45±10
Fasting glycerol (μmol/l)	118±9	0.03	155±20†	0.002	84±7†	121±7	NS	124±12†	0.01	84±7
Steady-state glycerol (μmol/l)	72±7	0.04	81±10*†	0.002	38±5	81±12	NS	92±15*†	0.002	33±4*

Values are mean±SEM or median (IQR)

[a] By Wilcoxon's test; [b] n=8; [c] n=12

*$p \leq 0.05$ vs obese controls and †$p \leq 0.05$ vs lean controls by Mann–Whitney U test

higher than in the lean group (Table 2). In the type 2 diabetic group, all patients were off glucose-lowering treatment and mean HbA_{1c} was 5.4±0.14 (36±2 mmol/mol) (p=0.03 vs baseline). While fasting NEFA were unchanged, steady-state NEFAm and fasting and steady-state glycerol were reduced at 1 year as compared with baseline (Table 2).

At 1 year, M (or M_{EE} or M_{FFM}) was significantly improved as compared with pre-surgery in non-diabetic and type diabetic patients. However, the values were still significantly lower than in lean controls. Likewise, insulin-mediated glucose oxidation was improved and lipid oxidation was reduced, as compared with pre-surgery, but both were still different from lean controls (Fig. 1a–c). On the other hand, EGP and R_aGly were reduced significantly, although still higher than in lean controls, while insulin-induced suppression of R_aGly was normalised (Table 3). At this time, M, EGP and R_aGly were at the same level as those of the obese controls.

When the mean group values for M, EGP and R_aGly were plotted against the corresponding mean BMI values (Fig. 2a–c), fasting EGP and R_aGly were positively related, and M was negatively related to BMI in a non-linear fashion. For all three metabolic variables, the values obtained after surgery, whether early or at 1 year, fell within or near the 95% CIs of the fit of the baseline measures of all 47 study participants. The same pattern was seen when regressing fasting EGP and R_aGly against fasting plasma insulin concentrations (Fig. 3a, b).

With regard to beta cell function in the type 2 diabetic group, fasting insulin secretion rate was significantly higher at baseline than that of 82 sex-, age- and BMI-matched historical controls (156 [117] median [IQR] vs 100 [IQR 48] pmol min^{-1} m^{-2}, p<0.01) [18], was marginally suppressed early after surgery (to 130 [IQR 100] pmol min^{-1} m^{-2}, p= NS) and was halved at 1 year (68 [IQR 40] pmol min^{-1} m^{-2}, p<0.03). In contrast, the baseline AIR (9 [IQR 36] pmol min^{-1} m^{-2} [mmol/l]$^{-1}$) was unchanged early after surgery (21 [IQR 42] pmol min^{-1} m^{-2} [mmol/l]$^{-1}$) and significantly improved at 1 year (67 [IQR 56] pmol min^{-1} m^{-2} [mmol/l]$^{-1}$, p=0.006) (ESM Fig. 4a–d).

Table 3 Glucose metabolism and lipolysis before and after RYGB

Variable	Non-diabetic patients					Diabetic patients				
	Pre-surgery	*p* value [a]	Early post	*p* value	1 year	Pre-surgery	*p* value	Early post	*p* value	1 year
M (μmol/min)	1,653 (953)	NS	1,880 (646)†	0.02	2,241 (737)	1,179 (1,176)	NS	1,988 (746)†	0.002	2,283 (953)*
M_{EE} (μmol/kJ)	293 (138)	NS	342 (173)†	0.002	470 (136)*†	176 (243)	NS	298 (205)†	0.002	516 (142)*†
M_{FFM} (μmol min^{-1} [kg FFM]$^{-1}$)	26.2 (13.5)	–	n.a.	0.002	38.5 (13.1)†	15.7 (22.4)	–	n.a.	0.002	42.2 (8.2)†
M_{FFM}/I (nmol min^{-1} [kg FFM]$^{-1}$ [pmol/l]$^{-1}$)	31 (25)	–	n.a.	0.002	75 (32)*†	23 (25)	–	n.a.	0.002	88 (35)*
EGP (μmol/min)	859 (274)	NS	834 (73)†	0.005	729 (300)†	844 (435)	NS	683 (194)	0.02	744 (185)†
EGP clamp (μmol/min)	−78 (240)	NS	−203 (208)	NS	32 (242)	−36 (233)	NS	−68 (261)	NS	60 (163)
EGP_{EE} (μmol/kJ)	150 (34)	NS	144 (17)	NS	166 (36)	138 (37)	NS	139 (35)	NS	158 (33)
R_aGly (μmol/min)	351 (114)	NS	372 (209)†	0.02	204 (90)†	349 (120)	NS	358 (175)†	0.02	254 (134)†
R_aGly_{FM} (μmol min^{-1} [kg FM]$^{-1}$)	4.24 (0.84)	–	–	NS	5.12 (3.17)	6.36 (1.42)	–	–	NS	9.31 (3.62)
R_aGly clamp (μmol/min)	154 (98)	NS	216 (111)†	0.03	96 (39)†	194 (134)	NS	259 (114)*†	0.003	73 (52)†
R_aGly suppression (%)	54 (9)	NS	44 (32)	0.04	59 (9)	39 (26)†	NS	28 (21)*†	0.002	69 (14)*

Values are median (IQR)

[a] By Wilcoxon's test

*$p \leq 0.05$ vs obese controls and †$p \leq 0.05$ vs lean controls, by Mann–Whitney U test

n.a, not applicable

Discussion

The major findings of these studies are: (1) tissue-specific insulin sensitivity and intracellular glucose disposition are improved in non-diabetic and type 2 diabetic patients 1 year after RYGB as a consequence of, and in approximate proportion to, the amount of weight lost; and (2) early after surgery, tissue sensitivity to insulin is little changed, but the sharp fall in insulin levels induced by the energy intake deficit reduces inhibition of lipolysis, whereby fatty substrates flood the circulation and force lipid oxidation. As a result, body fat begins to melt away and weight loss ensues. Importantly, this mechanism is not specific to surgery-induced energy intake restriction and is largely independent of insulin sensitivity. Therefore, the present studies, which are the first to use the clamp method to measure tissue-specific insulin resistance early and late after RYGB, do not support the hypothesis that RYGB impacts on insulin action prior to and independently of weight loss [26, 27]. Recently, Campos et al. [4], also using the clamp technique, found no modification in whole-body glucose uptake in non-diabetic individuals 14 days after RYGB, whereas 6 months later an improvement proportional to weight loss was seen. Our work extends this result to type 2 diabetic patients and identifies the tissues involved in the phenomenon (muscle, adipose tissue and liver).

Baseline The obese participants exhibited the full spectrum of metabolic abnormalities associated with obesity [28]. Thus, in comparison with lean controls, their whole-body insulin sensitivity was impaired, EGP was increased in absolute terms and lipolysis, as indexed by the systemic rate of glycerol appearance and reflected in circulating NEFA and glycerol levels, was enhanced in the fasting and insulinised state. Basal energy expenditure was increased and the oxidised fuel mix was shifted in favour of lipid at the expense of glucose. During the clamp, reduced glucose oxidation and lower non-oxidative glucose disposal contributed to insulin resistance. The defect in insulin-mediated glucose disposal was particularly severe in the type 2 diabetic group, in whom AIR was also severely compromised (ESM Fig. 4d), thereby contributing to hyperglycaemia.

In the baseline dataset, each of these metabolic variables was quantitatively related to BMI, in a direct fashion for EGP and R_aGly, and in a reciprocal fashion for M (Fig. 2). There was, however, one important difference between these variables. M was still significantly reduced in the obese (and reciprocally related to BMI) when expressed per kg FFM (or, equivalently, per kJ of basal energy expenditure), indicating that insulin action on glucose uptake was defective in each unit mass of metabolically active tissue. In fact, in the obese groups, the deficit in insulin-mediated glucose disposal averaged 34%, 40% and 57% of that in lean controls (in obese controls, and non-diabetic and type 2 diabetic surgical patients, respectively) when indexed as M total body, whereas it was markedly more pronounced when expressed per kg of FFM (M_{FFM}, 54, 59 and 75%) or as M_{FFM}/I (64, 72 and 80%). This finding confirms that in

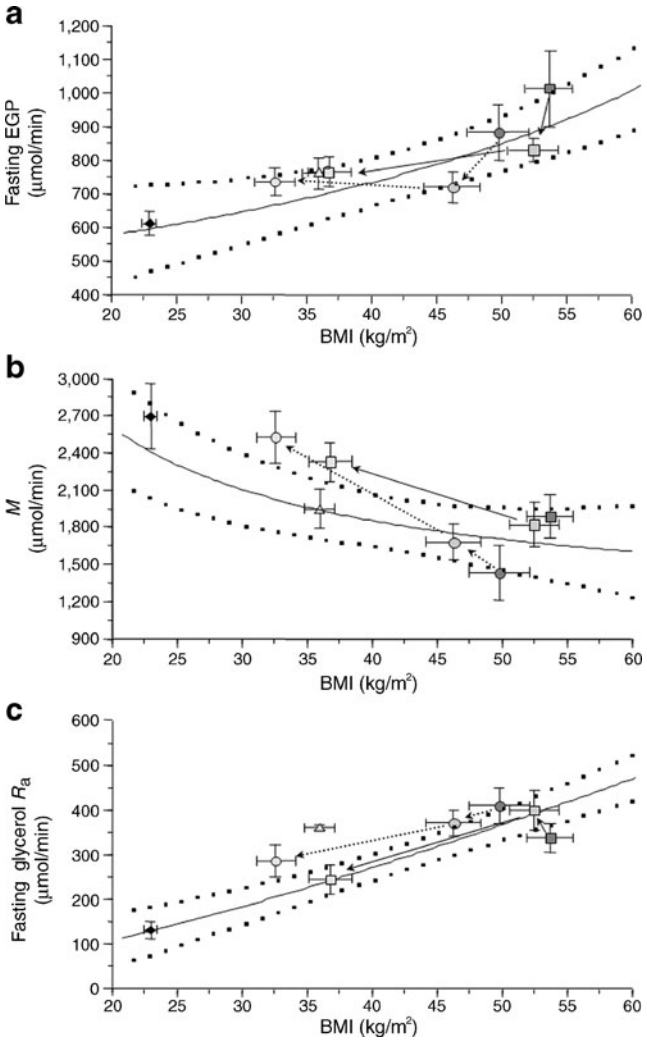

Fig. 2 Relationship between BMI and (**a**) fasting EGP (r=0.59, p< 0.0001), (**b**) (M) (r=−0.37, p<0.01) and (**c**) fasting lipolysis (as glycerol R_a) (r=0.79, p<0.0001) in the study participants. Continuous lines, best fit; dotted lines, 95% CI of best fit. Data obtained at baseline in the 47 study participants. Superimposed are mean±SEM values of the lean control (rhomboids), obese controls (triangles), and non-diabetic (squares) and type 2 diabetic (circles) patients undergoing RYGB. Arrows identify trajectories from baseline to 2 weeks to 1 year post-surgery

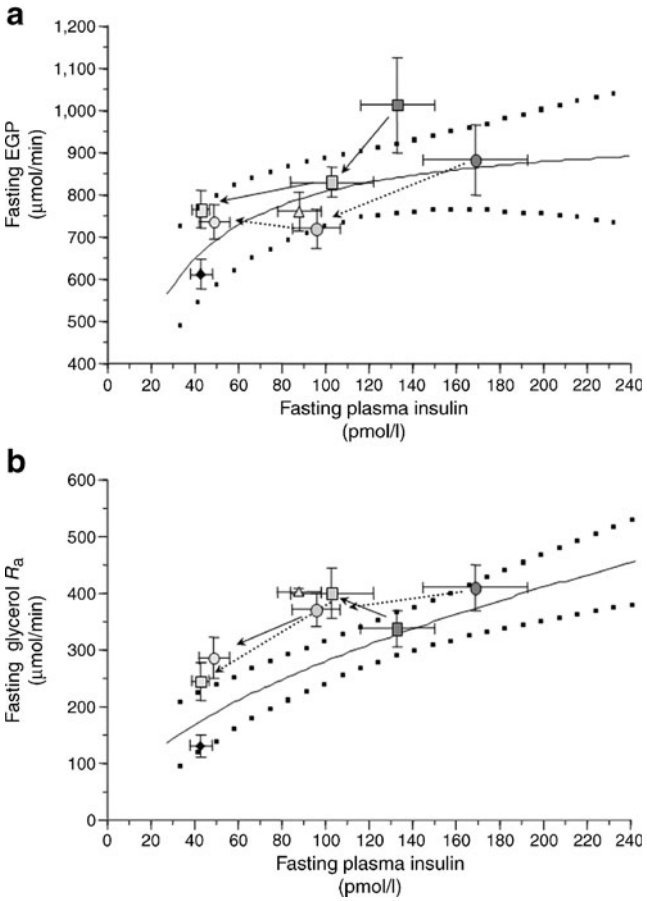

Fig. 3 Relationship between fasting plasma insulin and (**a**) fasting EGP (r=0.45, p=0.001) and (**b**) fasting lipolysis (as the glycerol R_a) (r=0.65, p<0.0001) in the study participants. Continuous lines, best fit; dotted lines, 95% CIs of best fit. Data obtained at baseline in the 47 study participants. Superimposed are mean ± SEM values of the lean control (black rhomboids), obese controls (triangles), and non-diabetic (squares) and type 2 diabetic (circles) patients undergoing RYGB. Arrows identify trajectories from baseline to 2 weeks to 1 year post-surgery

obesity, the expansion of lean body mass that accompanies fat accretion offers some compensation for whole-body insulin-mediated glucose disposal by providing an additional sink for circulating glucose, as shown in previous studies [29]. In contrast, in the obese groups, EGP was increased in fairly exact proportion to BMI, such that upon correcting for basal energy expenditure (or kg lean body mass), EGP no longer differed from that in controls (Fig. 2a). Because of the higher fasting plasma insulin concentrations (which normally restrain EGP), 'normal' EGP rates really reflect a state of hepatic insulin resistance, which increases along the trajectory calculated in Fig. 3a [30]. The same interpretation applies to lipolysis: enhanced in absolute terms, normal when expressed per kg of fat mass (Table 1), insulin-resistant when viewed in the context of prevailing insulin concentrations (Fig. 3b).

Early postsurgical period By 2 weeks after the operation, plasma glucose and insulin levels dropped, with no major change in M or EGP, and a large increase in circulating NEFA and glycerol levels, and in lipid oxidation rates, in the fasting state as well as during the clamp. This metabolic picture can be explained by the marked deprivation of energy intake that patients experience early after RYGB, which is also attested by the significant fall in basal energy expenditure [31]. The drastic effect of severe energy intake restriction on plasma glucose and insulin levels (both lowered), and on EGP has been observed previously. In seven diabetic patients receiving a 3,350 kJ/day for 7 days, Kelley et al. [32] reported a reduction in EGP concomitant with a fall in plasma glucose

and insulin concentrations. In 12 obese, insulin-treated diabetic patients subjected to 2 days of dieting at 1,883 kJ/day, Jazet et al. [33] found that EGP was significantly reduced, while peripheral insulin sensitivity and intramuscular insulin signalling were unchanged [34]. In a study in poorly controlled type 2 diabetic patients (HbA_{1c} ~10%), a low-dose overnight insulin infusion normalised EGP without changing peripheral insulin sensitivity as measured during an insulin clamp [35]. Thus, the likely sequence of metabolic events in our patients early after RYGB is that the energy intake deficit (estimated not to exceed 3,350 kJ/day) led to lower plasma insulin levels, which reduced inhibition of lipolysis [36], whereby lipids replaced carbohydrate as oxidative fuel and fat mass began to melt away. In the type 2 diabetic group, a large decrease in fasting plasma glucose was brought about by small but concomitant decrements in EGP and increments in M (Table 3), both possibly aided by relief of glucose toxicity. Viewed in the context of prevailing insulin levels, hepatic and adipose tissue insulin resistance were improved (Fig. 3a, b). With regard to beta cell function, AIR in type 2 diabetic patients was unchanged early after surgery, suggesting no acute influence of surgery on the beta cell response to glycaemia as such. However, we cannot exclude an acute effect of RYGB on the beta cell response to oral glucose or mixed meals, as this would be potentiated by incretin hormones, which are known to be increased after RYGB [9].

Follow-up at 1 year after surgery RYGB had induced the expected large decrease in body weight (averaging ~35% of initial weight) to similar degrees in non-diabetic and type 2 diabetic patients, which now matched that of the obese control group. At this time, peripheral insulin sensitivity was markedly improved; the improvement was significantly greater in the diabetic patients than in the non-diabetic group by all measures (M, M_{EE}, M_{FFM}, M_{FFM}/I, $p<0.04$ to $p<0.01$), presumably as a combined result of weight loss and removal of glucose toxicity. In fact, diabetes was nominally in remission in all patients and AIR was significantly better than at baseline (ESM Fig. 4d). However, peripheral insulin sensitivity was still lower than normal and, though marginally better than in the matched obese group, had improved essentially in proportion to the fall in BMI.

With regard to EGP and R_aGly, absolute values were significantly reduced as compared with baseline, but still higher than in lean controls and still proportionate to the BMI achieved.

In summary, in morbidly obese patients, RYGB produces metabolic effects on liver, adipose tissue and muscle insulin sensitivity, as well as on pattern of substrate utilisation. These effects are well explained by energy intake restriction and weight loss, the former prevailing early after surgery and the latter being dominant in the longer term. The current data showed no evidence of weight-independent effects on tissue insulin sensitivity such as those we and other members of our team have previously reported in patients undergoing malabsorptive bariatric surgery (biliopancreatic diversion) [37, 38].

Our study has limitations. First, given the complexity of the experimental procedures, the study groups were small. Second, not all RYBG patients have fully stabilised their energy balance even by 1 year after the operation; some may still be in mild energy deficit, thereby introducing some noise into the measurements. In all the type 2 diabetic patients in this study, whose diabetes was of relatively recent onset and mild in degree, there was remission at 1 year after surgery, a time at which abnormalities in insulin action persisted to an extent. Whether dysglycaemia or overt diabetes would recur, especially in patients regaining weight, remains to be explored in studies with a longer follow-up. Finally, the full impact of RYGB on the incretin system, especially on beta cell function, could not be explored because oral glucose or meal testing was not performed.

Acknowledgements This work was supported in part by a grant of the European Foundation for the Study of Diabetes (EFSD, Düsseldorf, Germany) and by the Italian Ministry for University and Research (MIUR 2007BRR57M-001). We wish to thank E. Barsotti and S. Burchielli (Department of Internal Medicine, University of Pisa, Italy) for their technical support. We are grateful to the patients who generously agreed to volunteer for these intense investigations.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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