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# Gastrointestinal Hormones, Intestinal Microbiota and Metabolic Homeostasis in Obese Patients: Effect of Bariatric Surgery

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**Abstract.** *Background/Aim: Bariatric surgery has proven efficacy in the modulation of a number of gut peptides that can contribute to improvement of diabetes and its associated metabolic changes. In order to evaluate dietary intake, nutritional assessment and plasma levels of gastrointestinal peptides, we enrolled severely obese patients before and after bariatric surgery. Patients and Methods: We evaluated food intake, plasma levels of peptide YY (PYY), glucagon-like peptide-1/2 (GLP-1/2), ghrelin (GHR), orexin (ORE) and cholecystokinin (CCK), body composition and fecal microbiota in 28 severely obese patients and 28 healthy normal-weight controls. All parameters were evaluated at 0 time and 6 months after bariatric surgery. Results: In obese patients we found a higher intake of nutrients, a decrease of free fat mass and an increase of BMI (body mass index), a significant decrease of GLP-1 and an increase of GLP-2, GHR and PYY with respect to controls, further increase in GLP-2, GHR and PYY, as well as increase over control values of GLP-1 after bariatric surgery. Obese individuals were found to harbor a community dominated by members of the Clostridial clusters XIVa and IV, whereas prominent bands after surgery were identified as Lactobacillus crispatus and Megasphaera elsdenii-related phylotype. Conclusion: The beneficial effects of bariatric surgery may at least in part be accounted for changes in circulating gastrointestinal (GI) peptides and fecal microbiota.*

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Gut releases a number of factors, such as peptides, lipids or starches that initiate a signalling to the brain for controlling food intake and triggering autonomic reflexes that regulate digestion (1, 2). These signal peptides, arising from the periphery, are classically divided into short-term 'episodic' signal peptides, such as cholecystokinin (CCK), peptide YY (PYY), ghrelin (GHR), glucagon-like peptides (GLP)-1 and -2 and orexin (ORE), rhythmically released in response to eating, and long-term 'tonic' signal peptides, such as insulin, leptin and adipokines, that are released in proportion to the amount of fat stores, reflecting the metabolic state (3, 4).

Obesity is a first magnitude health problem because of its increased prevalence in Western countries and the difficulties concerning both prevention and disease treatment. Severe obesity is associated with decreased life expectancy by 5-20 years (5). The effectiveness of behavioural, dietary and drug therapy in morbid obesity treatment is very limited, as well as the possibility to achieve sustained weight loss in morbidly obese patients (6, 7). Over the last years, bariatric surgery has provided interesting results, not only in achieving and maintaining appropriate weight loss but, most importantly, in ameliorating cardiovascular risk factors (8) and also in modulating plasma levels of gut peptides. For this reason, bariatric surgery represents the treatment-of-choice in selected morbid obese patients (6), being associated with long-term weight loss, improvement of cardiovascular profile, reversal of type 2 diabetes, and amelioration of quality of life (7).

The mechanisms underlying weight loss and metabolic changes associated with bariatric surgery have not been fully elucidated. Changes in rate of eating, gastric emptying, nutrient absorption and sensing, bile acid metabolism, gut neuroendocrine secretions, as well as microbiota may all be relevant (9). Microbial changes in the human gut were proposed as a possible cause of obesity (10, 11) and dietary

habits constitute a major factor influencing the diversity of human gut microbiota (12). A few studies in both humans and rats have reported that Roux-en-Y Gastric Bypass (RYGB) causes marked shifts in the distal gut microbiota (13-15). Such alteration of the gut bacteria population has been associated with decreased leptin levels in humans (15) and with global changes in fecal and urinary metabolites in rats (13). Liou *et al*. have demonstrated that fecal transplant of gut microbiota from RYGB-treated mice to germ-free mice results in weight loss and decreased fat mass, potentially due to altered microbial production of short-chain fatty acids, thus substantiating the hypothesis that beneficial effects of RYGB surgery are due at least in part to changes in the gut microbial community (16).

While the RYGB-associated modulation of gut peptides (GLP-1 and PYY) has been shown to contribute to the improvement of diabetes and appetite sensations, these specific modifications do not explain *per se* all the metabolic changes associated with these surgical interventions (17, 18).

The primary end-point of this prospective study was to evaluate the dietary intake, the nutritional status, as well as plasma levels of a number of gastrointestinal peptides that regulate food intake and fecal microbiota in severely obese patients and healthy non-obese control subjects. Also, as a secondary end-point, we evaluated whether bariatric surgery affected gastrointestinal (GI) peptides plasma levels and fecal microbiota.

## Patients and Methods

*Patients*. Between October 2010 and March 2011, 28 severely obese patients were referred to our Department for evaluation in view of bariatric surgery. Nineteen underwent biliointestinal bypass, whereas the remaining nine were excluded from surgery because of the following reasons: anatomical, as specified by the surgeon (n=1); elevated anaesthesiology risk, mainly related to respiratory or cardiac diseases (n=2); refusal to undergo surgery (n=2); psychiatric disorders (n=3); renal failure (n=1). We also studied 28 healthy normal weight controls, recruited from the outpatient gastroenterology unit with only reflux disease.

Informed written consent was obtained from all participants included in the study and all procedures performed in the study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The main demographic characteristics of patients evaluated are resumed in Table I.

To be eligible for the bariatric surgery, all patients had to have fulfilled the following criteria: a) morbid obesity (body mass index (BMI; in kg/m$^2$) >40) or severe obesity (BMI >35), at least one comorbidity factor (arterial hypertension, diabetes mellitus) for ≥5 years, and resistance to medical treatment; b) absence of medical or psychological contraindications for bariatric surgery; c) absence of current excessive drinking, as defined by average daily consumption of alcohol of 20 g/daily for women and 30 g/daily for men, and no history of past excessive drinking for a period >2 years at any time in the past 20 years; d) absence of long-term consumption of hepatotoxic drugs; e) negative screening for chronic liver diseases, including negative testing for hepatitis B surface antigen and hepatitis C virus antibodies, and other known liver diseases, such as Wilson's disease, hemochromatosis, α1-antitrypsin deficiency or autoimmune hepatitis; f) no malignant diseases.

At baseline, all patients were evaluated by a multidisciplinary team consisting of dieticians, gastroenterologists, psychiatrists, anaesthesiologist, cardiologist, endocrinologist and surgeons.

In severely obese patients who underwent surgery, all parameters were evaluated at 0 time (*i.e*. prior to surgery) and 6 months after bariatric surgery.

*Biochemical evaluation*. An overnight fast of at least 12 h preceded the insertion of an antecubital vein catheter for blood collection. The following clinical and biological features were assessed before surgery: BMI, blood pressure, aspartate aminotransferase (AST), alanine aminotransferases (ALT), gamma-glutamyltranspe-ptidase (γGT), prothrombin time, platelets, serum triglyceride, cholesterolemia, fasting blood glucose, fasting insulin. Insulin resistance was determined by the homeostatic model assessment method (HOMA) in which higher values of HOMA represent greater degrees of insulin resistance (19). The HOMA for insulin resistance was deduced in this model according to measurements of fasting glucose and insulin. Briefly, the equation is: HOMA=(insulin × glucose)/22.5, where insulin is expressed in μU/ml and glucose in mmol/l (19).

Diabetes, hypercholesterolemia and hypertriglyceridemia were defined as follows: fasting blood glucose >1.26 g/l, cholesterolemia >2.4 g/l and serum triglyceride >1.5 g/l.

*Nutritional assessment*. In all subjects, food intake was evaluated by an electronic program (WinFood, Medimatica s.r.l., Martinsicuro, Italy). On the basis of the quantities and qualities of consumed foods, the program elaborates the energy intake and the percentage of macronutrients and micronutrients and calculates the elements in each food. The complete elaboration of intakes shows the list of diet components, the ratio among components and calories, as well as the subdivision in breakfast, lunch and dinner. We recorded the food intake of a complete week, including working days and the weekend. The data were compared with the tables of food consumption and recommended dietary intakes of the Italian National Institute of Nutrition and Food Composition Database in Italy (20). Alcohol use was evaluated with a standardized pre-codified questionnaire (complete AUDIT test) (21). The quantity of daily alcohol intake was calculated based on a "drink" that corresponds to about 12 g of pure ethanol (22).

Anthropometric measurements obtained included body weight, body height, waist circumference (WC) and hip circumference. Body composition was evaluated by bioimpedance analysis (BIA 101S Akern, Florence Italy).

*Biliointestinal bypass*. The surgeon examined all patients and explained in detail the procedures of biliointestinal bypass (standardized information). The biliointestinal bypass described by Eriksson consisted of jejunoileostomy coupled with cholecystojejunal anastomosis (23).

All patients underwent a personal modification of the biliointestinal bypass (24). In brief, after identification of Treitz ligament, jejunum is sectioned 40 cm distally with a linear stapler. Then, a side-to-side anastomosis is performed between proximal

Table I. *Main demographic and biochemical findings of evaluated patients.*

| | Normal weight controls | | Obese patients | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Total number | 11 | 17 | 8 | 20 |
| Median age yrs | 45 | 33.5 | 50.5 | 45 |
| (range) | (34-56) | (21-50) | (39-55) | (26-63) |
| BMI (M±SD) | 25±1.4 | 21.7±1.5 | 54.3±18.5[1] | 48.6±8.1[1] |
| Diabetes mellitus, n (%) | 0 | 0 | 4 (50%) | 6 (30%) |
| Arterial hypertension, n (%) | 0 | 0 | 3 (37%) | 9 (45%) |
| HOMA (M±SD) | 2.4±1.1 | 2.6±1.2 | 24.2±13.1[2] | 23.3±14.4[2] |
| AST (IU/l; M±SD) | 14±3 | 16±2 | 20.6±8.4 | 23.8±10.3 |
| ALT (IU/l; M±SD) | 21±3 | 20±3 | 29.6±7.2 | 28.3±17.3 |
| γGT (IU/l; M±SD) | 19±3 | 18±6 | 46.3±38.3 | 58.8±114.8 |
| Cholesterol (mg/dl; M±SD) | 135±64 | 144±58 | 209±47.8 | 204±34.8 |
| Triglycerides (mg/dl; M±SD) | 108±15 | 93±18 | 149.4±42.2 | 212.1±295.3 |

[1]$p<0.01$ and [2]$p<0.05$ *vs*. normal-weight controls. BMI, Body mass index; M, mean; SD, standard deviation; ALT, alanine aminotransferases; AST, aspartate aminotransferases; γGT, gamma-glutamyltranspeptidase, HOMA, homeostatic model assessment; IU, international units; NAFLD, non-alcoholic fatty liver disease.

jejunum limb and ileum 40 cm proximally to ileo-cecal valve by means of a 60 mm endoscopic linear stapler, in order to create an 80 cm long alimentary and common tract. A side-to-side anastomosis between blind distal jejunal limb and gallbladder is then performed with a 45 mm endoscopic linear stapler, in order to switch bile directly in jejunum avoiding duodenal transit. Closure of enteric defects is made by a two-layer running suture. Peters' defect is then repaired to avoid internal hernia.

*Plasma levels of GI peptides*. Total GHR, GLP-1, GLP-2, ORE, PYY and CCK were measured with a commercially available kit by the quantitative enzyme immunoassay technique (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). This enzyme immunoassay kit is designed to detect a specific peptide and its related peptides, based on the principle of "competitive" enzyme immunoassay. In brief, the immunoplate is pre-coated with secondary antibody and the nonspecific binding sites are blocked. The secondary antibody can bind to the Fc fragment of the primary antibody (peptide antibody) whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in samples. The biotinylated peptide interacts with streptavidin-horseradish peroxidise (SA-HRP), which catalyzes the substrate solution. The yellow intensity is directly proportional to the amount of biotinylated peptide-SA-HRP complex, but inversely proportional to the amount of the peptide in standard solutions or samples. This is due to the competitive binding of the biotinylated peptide with the standard peptide or samples to the peptide antibody (primary antibody). The unknown concentration is determined by extrapolation to this standard curve. The unit of measure is ng/ml.

*Fecal microbiota analysis*. Fecal dominant bacterial community was investigated in obese subjects (n=11) before and after surgery by means of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. The Maxwell® 16 DNA Purification Kit and the Maxwell® 16 Instrument (Promega) were used to extract DNA from stool samples, according to the manufacturer's instructions. PCR amplification was performed using the universal bacterial primer pairs Hda1-GC/Hda2 and the thermo-cycling conditions reported by Walter *et al*. (25). PCR products were analysed by DGGE with the Ingeny PhorU apparatus (INGENY, Leiden, the Netherlands) using electrophoresis conditions as previously described (26). Dominant bands were excised from the gel, eluted in water, re-amplified with the original primer set and sequenced at BMR Genomics (University of Padova). Sequences were compared to the Ribosomal Database Project II (RDP II) database of 16S rRNA genes for species identification (http://rdp.cme.msu.edu/). Fingerprinting II (Bio-Rad Laboratories, Hercules, CA, USA) software was used to perform cluster analysis of DGGE bacterial fingerprint profiles with the unweighted pair group method using averages (UPGMA) based on the Dice index of similarity.

*Statistical analysis*. Continuous normally distributed variables were summarized as mean±standard deviation (SD) and categorical variables as frequency and percentage. The non-paired *t*-Test was used. Differences were considered significant for *p*-values inferior to or equal to 0.05. All analyses were performed by SPSS 12.0 version for WINDOWS (Microsoft, Seattle, WA, USA).

## Results

*Patients' characteristics*. Table I summarizes the main characteristics of the 28 obese patients and 28 healthy, non-obese controls. Surgery was performed in 19/28 patients with BMI >40 or severe obesity (BMI >35), with at least one comorbidity factor (arterial hypertension, diabetes mellitus) for ≥5 years, and resistance to medical treatment.

*Nutritional assessment*. Tables II and III, respectively, show the intake of macronutrients and micronutrients in patients and controls together with the daily amounts recommended in Italy. Obese pre-surgery patients, both males and females, showed significant increase of total calories, total proteins,

Table II. *Intake of macronutrients in study population (↓ ↑ indicate variations in respect to amounts recommended in Italy) (Mean±SD).*

| | | Normal weight controls | | Obeses pre-surgery | | Obeses post-surgery | |
|---|---|---|---|---|---|---|---|
| | Recommended | M | F | M | F | M | F |
| Total calories (kcal/day) | ♂: 2,000-2400 ♀: 1,800-2300 | 1,509±152 | 1,284±92 | 4,456±1570[1]↑ | 3,069±714[1]↑ | 4,248±1810[1]↑ | 3,179±624[1]↑ |
| Alcohol intake (g/day) | - | 14±11 | 8.6±3.1 | 28.3±56.2 | 0 | 16.2±39.4 | 0 |
| Total proteins (g/day) | 75 g | 69±6 | 58±21 | 180.3±39.2[1]↑ | 130.4±28.6[1]↑ | 176.4±41.8[1]↑ | 136.5±31.2[1]↑ |
| Total lipids (g/day) | 65 g | 56±7 | 45±15 | 150.1±35.7[1]↑ | 112.7±32.5[1]↑ | 170.2±24.9[1]↑ | 118±30.7[1]↑ |
| Saturated fatty acids (% of total ) | 7% | 17±3 | 14±4 | 33.8±20.3[1]↑ | 27.5±16.0[1]↑ | 36.2±24.1[1]↑ | 29.4±15.1[1]↑ |
| Polyunsaturated fatty acids (% of total ) | 18% | 8±2↓ | 5±2↓ | 9.7±3.4↓ | 7.7±2.3↓ | 8.9±2.4↓ | 8.0±2.9↓ |
| Monounsaturated fatty acids (% of total ) | 4% | 23±3↑ | 18±7↑ | 52±11[1]↑ | 42±10[1]↑ | 48±9[1]↑ | 46±12[1]↑ |
| Cholesterol (mg/day) | 255 mg | 247±71 | 161±54 | 504.7±97[1]↑ | 312.0±99.7[1]↑ | 485.2±88[1]↑ | 324.2±96.4[1]↑ |
| Total carbohydrates (g/day) | 290 g | 179±26↓ | 169±58↓ | 628±308[1]↑ | 409±120[1]↑ | 602±294[1]↑ | 441±136[1]↑ |
| Soluble carbohydrates (g/day) | 70 g | 53±10 | 56±23 | 72.5±34.3 | 79.6±46.0 | 70.4±28.2 | 74.2±49.0 |
| Amide (g/day) | 220 g | 110±22 | 99±36 | 508±268[1]↑ | 285±89[1]↑ | 512±274[1]↑ | 275±81[1]↑ |
| Fiber (g/day) | 23 g | 13±3 | 13±5 | 34±13[1]↑ | 20±4[1] | 36±16[1]↑ | 20±3[1] |

[1]$p<0.05$ *vs*. normal weight controls.

Table III. *Intake of micronutrients in study population (↓ ↑ indicate variations in respect to amounts recommended in Italy) (Mean±SD).*

| | | Normal weight controls | | Obeses pre-surgery | | Obeses post-surgery | |
|---|---|---|---|---|---|---|---|
| | Recommended | M | F | M | F | M | F |
| Zinc (mg/day) | 7 | 7.9±0.9 | 7.9±2.9 | 18.6±1.3[1]↑ | 13.9±4.0[1]↑ | 16.4±1.1[1]↑ | 13.5±3.7[1]↑ |
| Folic acid (μg) | 200 | 108.1±25.6↓ | 104.8±57.3↓ | 305.4±87.3[1]↑ | 229.6±79.8[1] | 324.2±89.4[1]↑ | 238.1±81.2[1] |
| Niacin (mg) | 14 | 13.7±5.8 | 11.7±4.0 | 39.3±7.1[1]↑ | 28.5±8.7[1]↑ | 40.2±7.0[1]↑ | 29.2±8.6[1]↑ |
| Thiamine (mg) | 0.9 | 0.9±0.1 | 0.7±0.2 | 2.4±0.7[1]↑ | 1.8±1.0[1]↑ | 2.3±0.6[1]↑ | 1.9±1.1[1]↑ |
| Vitamin A (μg) | 600 | 586.7±165.5 | 739.3±327.9 | 1057.6±379.2[1]↑ | 894.3±258.8 | 1068±381[1]↑ | 924.2±261 |
| Vitamin E (mg) | 8 | 5.1±1.8↓ | 4.4±1.6↓ | 11.4±3.3[1] | 10.2±3.1[1] | 11.2±3.6[1] | 11±4.2[1] |
| Calcium (mg/day) | ♂:1200 ♀:1500 | 595.1±141.4↓ | 672.2±231.3↓ | 1019.7±555.8[1] | 759.3±413.9↓ | 1017±561.1[1] | 764±408.1↓ |
| Iron (mg/day) | 18 | 8.4±2.4↓ | 7.8±2.1↓ | 20.1±5.5[1] | 13.8±2.7[1]↓ | 20.3±4.9[1] | 12.9±2.9[1]↓ |
| Riboflavin (mg/day) | 1.2 | 1.1±0.1 | 1.6±1.2 | 2.7±0.4[1]↑ | 2.2±0.4[1]↑ | 2.9±0.7[1]↑ | 2.3±0.6[1]↑ |
| Vitamin B6 (mg/day) | 1.1 | 0.7±0.2 | 0.7±0.3 | 1±0.4[1] | 0.6±0.4 | 1.1±0.3[1] | 0.8±0.3 |
| Vitamin C (mg/day) | 70 | 51.6±29.0 | 104.4±45.1 | 135.6±51.9[1]↑ | 136.1±41.3↑ | 148.2±54.8[1]↑ | 138.2±40.3↑ |
| Vitamin D (μg/day) | 10 | 9.1±8.2 | 8.4±7.2 | 5.5±2.3 | 5.2±2.4 | 5.7±2.1 | 5.3±2.1 |

[1]$p<0.05$ *vs*. normal weight controls.

lipids (saturated, monounsatured fatty acids and cholesterol) and complex carbohydrates (starch) (Table II) with respect to normal weight controls and to the amounts recommended in Italy (20). Also, concerning the micronutrients, obese pre-surgery patients showed significant variation of some minerals, both with respect to normal weight controls and to the amounts recommended in Italy (Table III).

*Effects of bariatric surgery on anthropometric and biochemical measurements*. None of the 19 patients (15 women and 4 men with a mean age of 45±10.1 years) had postoperative complications. During the follow-up period, the mean BMI fell from 49.8±10.6 to 40.8±6.6 kg/m2 ($p$=0.002) and the mean percentage of excess weight loss was 19.6%±7.0%. Six months after surgery, waist circumference ($p$=0.002) and waist-to-hip ratio ($p$=0.005) also showed significant improvement. Parameters for metabolic syndrome ($p$=0.003) and insulin resistance ($p$=0.002) significantly improved after surgery. Biochemical improvement was found in serum levels of ALT ($p$=0.002) and γGT ($p$=0.005) but not in AST ($p$=0.3). Other biochemical variables, including fasting glucose, triglyceride, cholesterol, LDL-cholesterol and

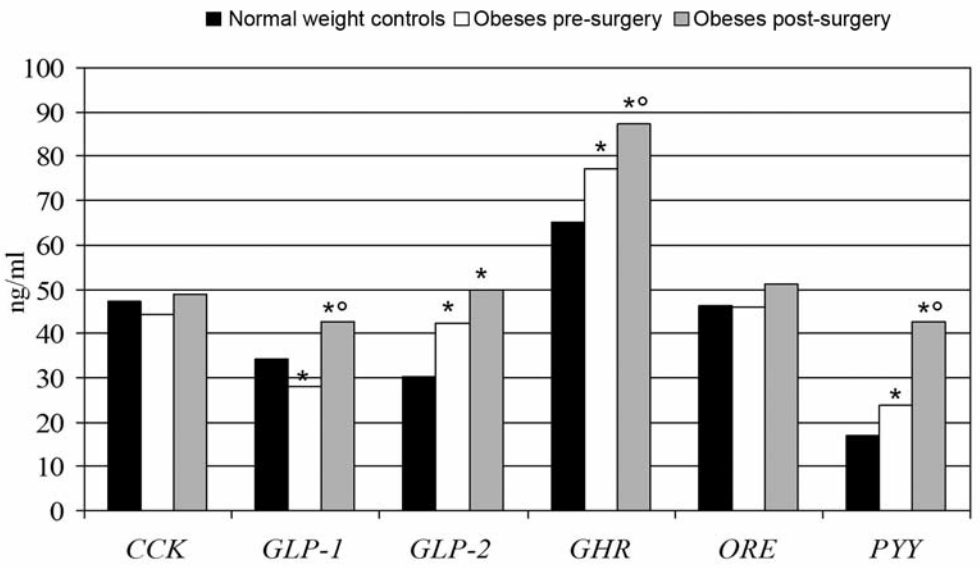


Figure 1. *Serum levels of a number of gastrointestinal hormones in obese patients before and after bariatric surgery (ng/ml; M±SD). CCK: Cholecystokinin; GLP: glucagon-like peptide; GHR: ghrelin; ORE: orexin; PYY: peptide YY. *p<0.05 vs. normal weight controls and °p<0.05 vs. obese pre-surgery patients. Black, white and gray bars represent normal weigh controls, obese pre-surgery patients and obese post-surgery patients, respectively.*

the HDL-cholesterol levels, were significantly ameliorated ($p<0.05$ *vs*. basal values; data not shown). In particular, preoperatively, all patients fulfilled the criteria for insulin resistance and, postoperatively, all patients normalised their HOMA value. Plasma levels of fasting glucose decrease was also normalized in all ten diabetic patients at 6 months after surgery. This reduction was observed when patients were still obese (BMI>30 kg/m$^2$). After bariatric surgery all diabetic patients exhibited normalised fasting glycemia.

Twelve patients who were hypertensive and hypercholesterolemic before surgery showed normalised their blood pressure and cholesterol levels after surgery. Moreover, all hypertriglyceridemic patients preoperatively exhibited normalised parameters after surgery.

*Gut hormone levels*. Preoperative fasting GHR levels were increased in obese pre-surgery patients ($p<0.05$ *vs*. controls) (Figure 1); postoperatively, fasting GHR levels significantly increased at 6 months ($p<0.05$ *vs*. normal weight controls and obese pre-surgery patients).

Fasting preoperative concentrations of GLP-1 were decreased ($p<0.05$) with respect to normal weight controls; by contrast, postoperatively, we observed an increase in GLP-1 levels ($p<0.05$ *vs*. normal weight controls and obese pre-surgery patients) over normal weight controls values (Figure 1).

PYY was increased in obese pre-surgery patients ($p<0.05$) *versus* normal weight controls; postoperatively, we observed a further increase in PYY levels ($p<0.05$ *vs*. normal weight controls and obese pre-surgery patients).

CCK and ORE levels were comparable in all groups with no significant differences (Figure 1).

GLP-2 was increased in obese pre-surgery patients ($p<0.05$) *versus* normal weight controls, while, postoperatively, we observed a further increment in GLP-2 levels ($p<0.05$ *vs*. normal weight controls and obese pre-surgery patients).

*Fecal microbiota*. Visual comparison of DGGE banding patterns indicated that the fecal bacterial profiles were highly heterogeneous (Figure 2a) and UPGMA analysis confirmed that the similarity between two distinct profiles from both pre- and post-surgery obese subjects was not higher than 65% (Figure 2b). Notably, there was a higher variation in DGGE profiles between duplicates obtained from the same subject before and after surgical intervention, than between different subjects. More specifically, the similarity of DGGE profiles varied from 50 to 65% among pre-surgery patients (with the exclusion of one patient), whereas post-surgery profiles displayed a slight lower similarity among each other, between 30% and 65% with the exception of two individuals (Figure 2b). The most intense and frequent bands in fecal samples were sequenced and the obtained sequences were compared to those available in the Ribosomal Database Project II database; sequence similarities are presented in Table IV. The prevalent bands in the DGGE profiles of obese subjects before surgery were identified as *Butyrivibrio fibrisolvens* (band 1, RDP score 0.925), *Roseburia hominis/faecis* (band 2, RDP score 1.00) *Dorea longicatena* (band 3, RDP score 1.00) *Blautia* sp./*Ruminococcus* sp.

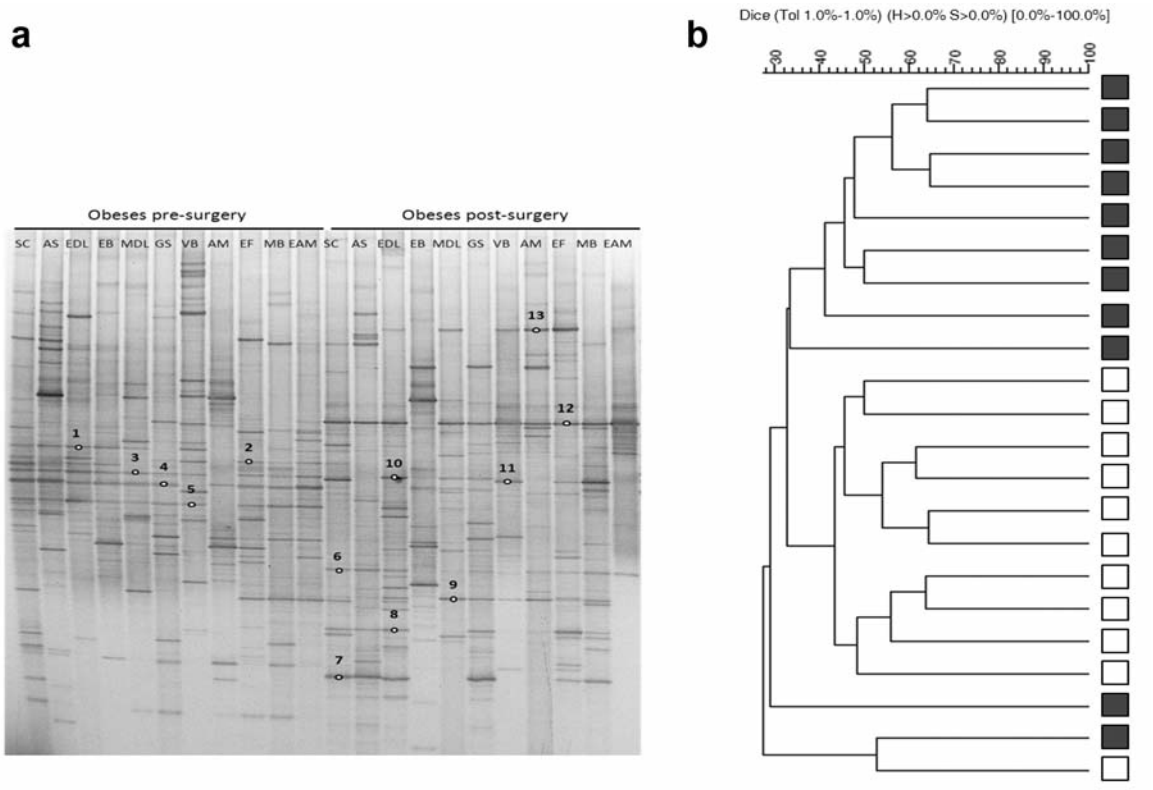


Figure 2. *DGGE analysis of fecal bacterial communities in obese individuals (n=11) before and after biliointestinal bypass. Bands subjected to re-amplification and sequencing are labeled with dot and number and correspond to the amplicon identities shown in Table IV (a). UPGMA dendrogram based on Dice similarity matching index produced from DGGE band patterns. White squares indicate fingerprints from pre-surgery samples, grey squares represent post-surgery samples (b).*

(band 4, RDP score 1.00) and *Ruminococcus obeum* (band 5, RDP score 0.933). All these bands were not consistently found, *i.e.* they became less intense or even disappeared in the profiles of patients after biliointestinal bypass. In comparison to pre-operative condition, band marked with number 12 in Figure 2a became prevalent in the DGGE profiles of all post-surgery obese patients, except for EB; sequence analysis indicated that this fragment had a 100% similarity (RDP score 1.00) with *Lactobacillus crispatus*. The fecal microbial profiles of patients revealed three bands migrating at different positions of the gel; nevertheless, the corresponding sequences were all related to *Megasphaera elsdenii* (bands 6, 7 and 8; Figure 2a). Six profiles out of 11 post-surgery obese fingerprintings showed a strongly marked band, numbered with 13, that displayed the highest similarity with *Streptococcus* spp., (RDP score 0.932). Analysis of a third band, indicated with number 9 in Figure 2a, seemed to be representative in most of the subjects after bariatric surgery; however, the sequence obtained for this band was of low quality and did not allow for consistent phylogenetic assignment.

## Discussion

Bariatric surgery has proven effective for achieving sustained weight loss in obese patients and is thought to be an attractive option in reversing many metabolic risk factors (27).

The present prospective study evaluated the plasma levels of a number of GI peptides that regulate food intake in obese patients to further elucidate their modifications before and after bariatric surgery. Many studies have shown that the variation of serum concentration of GI hormones (in particular, GLP-1 and PYY) plays an important role in body weight loss after bariatric surgery (18). All patients did not change their eating style and maintained their food intake after biliointestinal bypass.

The mechanism by which the GI hormones can interfere with the reduction of body weight after bariatric surgery is not yet known.

GLP-1 is an incretin hormone secreted by enteroendocrine L-cells of the small intestine targeting pancreatic β-cells to release insulin and reduce glucagon production in response to food intake (28, 29). In our study, bariatric surgery

Table IV. *Closest relatives of the sequenced DGGE bands, as indicated in Figure 2a, in the RDP II database.*

| Band no. | Closest match | RDP score |
|---|---|---|
| 1 | *Butyrivibrio fibrisolvens* | 0.925 |
| 2 | *Roseburia hominis/faecis* | 1.00 |
| 3 | *Dorea longicatena* | 1.00 |
| 4 | *Blautia* sp./*Ruminococcus* sp. | 1.00 |
| 5 | *Ruminococcus obeum* | 0.933 |
| 6 | *Megasphaera elsdenii/hominis* | 0.921 |
| 7 | *Megasphaera elsdenii/hominis* | 0.921 |
| 8 | *Megasphaera elsdenii* | 0.730 |
| 9 | n.i. | |
| 10 | *Prevotella ruminicola* | 0.898 |
| 11 | *Streptococcus gallolyticus/ equinus/pasteuri/lutetiensis* | 1.00 |
| 12 | *Lactobacillus crispatus* | 1.00 |
| 13 | *Streptococcus* sp. | 0.932 |

n.i., Not identified; RPD, Ribosomal Database Project II.

significantly increased plasma levels of GLP-1. This might be accounted for a more rapid transit of nutrients at the distal ileum, which might stimulate the secretion of hormones, such as GLP-1 (30).

Bariatric surgery is considered to be a potentially effective procedure to achieve a remission of type 2 diabetes mellitus in obese patients (31). In our surgical patients, diabetes was in remission and this evidence is in accordance with a recent study showing that the administration of GLP-1 was highly effective in the treatment of type 2 diabetes, thus producing significant improvements in glycemic profile, insulin sensitivity and performance of the β-cells, as well as in body weight reduction (32). This benefit on glucose metabolism appears in the early postoperative period before any significant loss of weight (31, 33). This observation has not been described after performing a restrictive technique, such as gastric banding (34, 35). One of the mechanisms proposed to explain the glycemic control after bariatric surgery is the changes in GI hormone levels (36, 37). This hypothesis is supported by our study. In fact, bariatric surgery was associated with early improvement in glucose metabolism, already 3 months after surgery, when patients are still obese (BMI ≥30 kg/m$^2$), and glucose levels remained normal at least 6 months after surgery. In addition, HOMA values were normalised in all patients 6 months postoperatively. The improvement in insulin sensitivity, associated with weight loss, is a well-known effect (38). A reason for the improved glycemic control could be related to changes in the secretion of GI hormones involved in insulin secretion and/or action (39). Changes in GLP-1 appear to be critical for improving the response to insulin. The GLP-1 release by L cells of small intestine and colon (40) is triggered by the arrival of food into the distal intestine and acts on pancreatic β-cells stimulating the release of insulin (41). Preoperatively, GLP-1 levels were low in obese patients. However, after surgery, there was an increase in postprandial GLP-1, which cannot only be justified by caloric restriction, since its secretion depends on the arrival of food into the distal small bowel. Low levels of GLP-1 detected in obese patients may reflect the state of functional deficiency that contributes to poor glycemic control in these patients. An increase in postprandial GLP-1 level, in addition to its incretin effect, acts as a signal of satiety promoting weight loss. The higher postprandial secretion of GLP-1 in biliointestinal bypass patients could be explained by the earlier arrival of nutrients to the ileum.

PYY is an anorexigenic neuropeptide with satiating action (4, 42). In our obese patients, PYY levels were lower than in controls. Postoperatively, our patients displayed an increase in plasma PYY concentrations, at least in part explained by weight loss and improved glycemic control. Our data are in agreement with previous studies showing augmented postprandial PYY levels after biliointestinal bypass (43, 44). Moreover, an increased PYY, at 3 and 12 months after laparoscopic sleeve gastrectomy, has recently been reported (45).

The gastric peptide GHR is released from X-cells during the interdigestive period and plasma concentrations decrease following a meal (46, 47). Administration of GHR accelerates gastric emptying, stimulates food intake, decreases energy expenditure and increases adiposity (48, 49). Also, GHR has been linked to adaptation to prolonged dietary restriction (50). GHR has several diabetogenic effects with the inhibition of insulin being the most relevant (51). In our study, GHR levels were found to be increased after biliointestinal bypass. A number of studies suggests that there are significant differences between laparoscopic sleeve gastrectomy and other bariatric procedures like the Roux-en-Y gastric bypass with the values of fasting GHR being significantly lower in the laparoscopic sleeve gastrectomy group at 3 and 12 months postoperatively (43, 45, 52). It appears that our results are linked to the intact stomach, a unique feature of biliointestinal bypass among all bariatric procedures.

Recent articles have shown that GLP-1 receptor agonists, such as exenatide and liraglutide, reduced body weight, blood pressure and improved lipid profile; in addition, the incretin family is gaining even more ground in the treatment of obesity (53).

In this study, all our patients showed a significant improvement in blood pressure levels, as well as in hypercholesterolemia and hypertriglyceridemia, six months after surgery. We also found significant improvements in serum levels of ALT and γGT (data not shown) and this is in agreement with several studies showing a considerable amelioration of liver function following bariatric surgery-induced weight loss (27, 54).

Consistently with previous findings, PCR-DGGE analysis of fecal samples indicated a dramatic alteration of bacterial community for obese patients who underwent biliointestinal bypass, as compared to pre-intervention asset. The fecal microbiota of pre-surgery obese patients was dominated by members of *Roseburia*, *Blautia*, *Ruminococcus* and *Dorea genera*, belonging to the Clostridial clusters XIVa and IV, and possessing the ability to ferment a large variety of carbohydrates. The main products of their fermentative metabolism are either butyrate, as for *Roseburia intestinalis*, or mainly acetate, as for *Ruminococcus obeum*. Such results are in line with other studies showing that the microbiome of genetically obese mice is enriched with genes related to the production of acetate and butyrate (55). Furthermore, post-intervention fecal profiles displayed a major band, identified as *Lactobacillus crispatus*. This bacterium, together with *Streptococcus* spp., whose sequences were also detected in post-surgery individuals, represents the major lactic acid producing bacteria inhabiting the rumen and the distal GI tract of mammals. It is likely that an increased amount of fermentable carbohydrates reaching the distal part of the intestine after surgery is able to boost the growth of such microorganisms. The second major bacterial population found in post-surgery obese subjects is related to *Megasphaera elsdenii*, which is known to be the most important lactate-utilizing bacterium in the rumen. Recently, it has been suggested that utilization of lactate by *Megasphaera* sp. may have a similar function in the human gut, *i.e.* reducing lactate toxicity by producing mainly propionate, followed by acetate and butyrate (56). It is conceivable that the microbial compositional differences observed in biliointestinal bypass subjects reflect different metabolic pathways and consequently influence the profiles of fermentation end products. This represents an interesting hypothesis in the light of increased evidence pointing at a role of short-chain fatty acids as signaling molecules involved in gut hormones production (57-60). Indeed, our results provide preliminary insight into the association between gut microbial community changes and biliointestinal bypass. Undoubtedly, further investigation is needed to highlight the impact of these changes on gut hormone modulation and energy metabolism in obesity.

Our current study has some limitations. First, the present results should be interpreted taking into account the small number of patients included. Therefore, additional studies in a larger population are needed to confirm these preliminary findings. On the other hand, more pure nutrient sources or different volumes, as well as solid foods, were not tested; hence, hormonal responses and changes to these challenges may be assessed in future studies relating, or not, to longer postoperative periods. Finally, the efficacy of bariatric surgery should be compared with other weight reduction programs, such as diet or exercise.

In summary, the present study demonstrated that severely obese patients show a significant change in the serum levels of a number of peptides presiding over metabolic homeostasis and in the composition of intestinal microbiota, as compared with healthy non-obese subjects. Moreover, bariatric surgery is significantly effective in decreasing body weight and controlling metabolic homeostasis, effects that are associated with changes in serum profiles of GLP-1, GLP-2, GHR and PYY, as well as in fecal microbiota composition. We hypothesize that these changes may account, at least in part, for the beneficial effects of bariatric surgery.

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