

Cholesterol Metabolism After Bariatric Surgery in Grade 3 Obesity

Differences between malabsorptive and restrictive procedures

ALBERTO BENETTI, MD^{1,2}
MARINA DEL PUPPO, PHD³
ANDREA CROSIGNANI, MD^{1,2}
ANNAMARIA VERONELLI, MD^{1,2}
ENZO MASCI, MD^{1,2}

FRANCESCA FRIGÈ, BSC^{1,2}
GIANCARLO MICHELETTI, MD^{4,5}
VALERIO PANIZZO, MD⁵
ANTONIO E. PONTIROLI, MD^{1,2}

OBJECTIVE—Malabsorptive bariatric surgery (biliopancreatic diversion and biliointestinal bypass [BIBP]) reduces serum cholesterol levels more than restrictive surgery (adjustable gastric banding [AGB]), and this is thought to be due to greater weight loss. Our aim was to evaluate the changes of cholesterol metabolism induced by malabsorptive and restrictive surgery independent of weight loss.

RESEARCH DESIGN AND METHODS—In a nonrandomized, self-selected, unblinded, active-comparator, bicenter, 6-month study, glucose metabolism (blood glucose and serum insulin levels and homeostasis model assessment of insulin resistance [HOMA-IR] index) and cholesterol metabolism (absorption: serum campesterol and sitosterol levels; synthesis: serum lathosterol levels; catabolism: rate of appearance and serum concentrations of serum 7- α - and serum 27-OH-cholesterol after infusions of deuterated 7- α - and 27-OH-cholesterol in sequence) were assessed in grade 3 obesity subjects undergoing BIBP ($n = 10$) and AGB ($n = 10$). Evaluations were performed before and 6 months after surgery.

RESULTS—Subjects had similar values at baseline. Weight loss was similar in the two groups of subjects, and blood glucose, insulin levels, HOMA-IR, and triglycerides decreased in a similar way. In contrast, serum cholesterol, LDL cholesterol, non-HDL cholesterol, serum sitosterol, and campesterol levels decreased and lathosterol levels increased only in BIBP subjects, not in AGB subjects. A significant increase in 7- α -OH-cholesterol occurred only with BIBP; serum 27-OH-cholesterol decreased in both groups.

CONCLUSIONS—Malabsorptive surgery specifically affects cholesterol levels, independent of weight loss and independent of glucose metabolism and insulin resistance. Decreased sterol absorption leads to decreased cholesterol and LDL cholesterol levels, accompanied by enhanced cholesterol synthesis and enhanced cholesterol catabolism. Compared with AGB, BIBP provides greater cholesterol lowering.

Diabetes Care 36:1443–1447, 2013

The epidemic of obesity has led to a dramatic increase of the number of bariatric procedures performed worldwide (1). Bariatric procedures are commonly divided into restrictive (adjustable gastric banding [AGB], vertical-banded gastroplasty, and sleeve gastrectomy), malabsorptive (biliopancreatic diversion

[BPD] and biliointestinal bypass [BIBP]), and mixed procedures (gastric bypass and Roux-en-Y gastric bypass [RYGB]) (1–3). Malabsorptive procedures are associated with a far greater weight loss than restrictive procedures and with quicker metabolic changes, namely drop of blood glucose levels (2).

Malabsorptive surgery also reduces serum cholesterol levels; in fact, it was originally intended for the treatment of hyperlipidemias and was highly effective even in patients without morbid obesity (the Program on Surgical Control of Hyperlipidemias study) (4). Malabsorptive surgery is more effective than restrictive surgery in reducing cholesterol levels (5), and our group has shown that by 1 year after surgery, both BPD and BIBP reduce cholesterol levels more than AGB, and this effect is associated with greater weight loss (6,7). In addition, the cholesterol decrease correlates with loss of fat mass (7). Therefore, the main factor considered responsible for decreased cholesterol levels is weight loss.

However, up to 6 months, weight loss is similar with BIBP and AGB (7), and this led us to hypothesize that it was possible to study cholesterol absorption, as well as other aspects of cholesterol metabolism, independently of weight loss.

Therefore, we planned a study on the intestinal absorption of sterols, cholesterol synthesis, and cholesterol catabolism via synthesis of biliary acids in obese subjects before and 6 months after BIBP or AGB.

RESEARCH DESIGN AND METHODS

Bariatric surgery (BIBP and AGB) is routinely performed at the obesity centers of the institutions participating in this study. Patients are eligible for bariatric surgery if they meet the following criteria: 18–65 years of age and a BMI >40.0 kg/m² alone or a BMI >35.0 kg/m² in the presence of comorbidities, plus a history of at least two previous dietary attempts followed by a relapse. The clinical protocol includes a medical history, physical and biochemical evaluation, and a psychological and psychiatric evaluation performed to verify indications, contraindications, and safety of surgical procedures (3,8). For all subjects undergoing BIBP or AGB, after surgery the suggested diet is 970 and 1,090 kcal per day in women and men, respectively; iron is supplemented on the basis of blood examinations performed during

From the ¹Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy; the ²Dipartimento di Medicina Interna, Ospedale San Paolo, Milan, Italy; the ³Dipartimento di Medicina Sperimentale, Università degli Studi di Milano Bicocca, University of Milan Bicocca, Milan, Italy; the ⁴Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano, Milan, Italy; and ⁵Istituto Nazionale per la Chirurgia dell'Obesità and Unità Operativa di Chirurgia Generale, Istituto Clinico Sant'Ambrogio, Milan, Italy.

Corresponding author: Antonio E. Pontiroli, antonio.pontiroli@unimi.it.

Received 27 August 2012 and accepted 6 November 2012.

DOI: 10.2337/dc12-1737

© 2013 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

the 2nd month. The diet includes 48% carbohydrates (starch or bread), 33% proteins (fat-free parts of different animals and fishes), and 19% lipids (olive oil); sweets, cakes, sweetened drinks, alcohol, and animal lipids are forbidden. All foods have to be cooked without oil, butter, or other lipids; in addition, BIBP patients receive vitamin D3 at discharge (two administrations per month), vitamin B12 (5,000 units once per month), and oral antidiarrheal drugs (diphenoxylate or loperamide) when there are >10 bowel movements per day (3,8).

Study protocol

This was a nonrandomized, self-selected, unblinded, active-comparator, bicenter, 6-month study. The whole protocol was approved by the local ethics committees. The subjects, all Caucasian, were informed about the nature and aims of the cholesterol metabolism evaluation protocol, and they signed a written informed consent. The choice of surgical procedure was made by the patient together with the surgeon, after a full explanation of the risks and possible benefits of each procedure. In total, 10 subjects underwent BIBP and 10 underwent AGB.

After all interventions, subjects were re-evaluated by a dietitian and physician at 2-week intervals for 2 months and then monthly for up to 6 months, as previously described (3,8). At baseline and 6 months after surgical procedures, the subjects were studied in the early morning in a recumbent position and after an overnight fast. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively.

Blood drawing and isotope infusion

A venous line was used for blood sampling, and another venous line on the opposite arm was used for infusions. Both venous lines were kept patent by a slow 0.9% NaCl saline drip.

After a blood drawing for metabolites, sequential infusions of deuterated oxysterols were performed between 9:00 A.M. and 2:00 P.M., according to a previously published protocol (9). Serum samples were obtained from blood collected during the sequential infusions of the two hydroxysterols at 0, 60, 80, 100, and 120 min. Plant sterols (campesterol and sitosterol) were measured as biomarkers of cholesterol absorption, and lathosterol as a marker of cholesterol synthesis (10). 7- α -OH-cholesterol and 27-OH-cholesterol are markers of bile acid metabolism, the

former of direct intrahepatic metabolism and the latter of mainly extrahepatic catabolism (9); bile acid synthesis accounts for >95% of cholesterol catabolism per day (9).

Laboratory assays

Plasma glucose was measured by the glucose oxidation method (YSI, Inc., Yellow Springs, OH). Serum insulin was assayed by microparticle enzyme immunoassay (Abbott Laboratories, Pasadena, CA) (8). Total cholesterol, HDL cholesterol, and triglyceride levels were assayed by automated enzymatic methods (Roche, Basel, Switzerland). LDL cholesterol was calculated using the Friedewald equation (11). Non-HDL cholesterol was calculated as total cholesterol – HDL cholesterol (12). The homeostasis model assessment of insulin resistance [HOMA-IR] index was calculated as blood glucose (mmol/L) \times insulin (μ U/mL) \times 22.5⁻¹ (13).

Quantitative evaluation of lathosterol, campesterol, and β -sitosterol in serum was performed by a gas-liquid chromatography/mass spectrometry (GC/MS)-based technique as previously described (14). For determination of hydroxysterol serum concentrations, after the addition of 250 ng 19-OH-cholesterol as internal standard to 0.2 mL serum, samples were hydrolyzed and extracted (15). The organic phase was evaporated to dryness under a stream of nitrogen and purified

by solid-phase extraction. The fraction containing the hydroxysterols was dried and treated with trimethylsilylimidazole: piperidine (1:1) before analysis by GC/MS.

Deuterated oxysterol infusions

Deuterated (d6) 7- α -OH-cholesterol was obtained by chemical synthesis from deuterated (d6) cholesterol, according to previously described methods (15), and deuterated 27-OH-cholesterol was prepared by synthesis from kryptogenin, using the Clemmensen reduction method, as previously described (16). Homogeneous suspensions of the sterols in 5% human albumin were prepared on the same day of the infusion experiment (final concentration of 3 and 6 mg/mL of deuterated 7- α - and 27-OH-cholesterol, respectively) and filtered through a 0.22- μ m Millipore membrane immediately before infusion, under a laminar flow hood (14). Serum samples, obtained from blood collected during the sequential infusions of the two hydroxysterols (see above), were analyzed for deuterium enrichment of 7- α - and 27-OH-cholesterol using GC/MS on extracted oxysterols as previously reported (14). The *in vivo* rate of appearance of the two hydroxysterols was calculated as milligrams per hour from the deuterated/natural sterol ion ratio and infusion rate using the equation described for the steady state by Duane and Javitt (17).

Table 1—Clinical and metabolic details of obese subjects in the study, divided by surgical technique

	BIBP (n = 10)	AGB (n = 10)
Age	45.1 \pm 1.59	47.5 \pm 2.04
Sex (male/female)	3/7	3/7
BMI (kg/m ²)	45.9 \pm 0.56	46.2 \pm 2.05
Blood glucose (mg/dL)	96.6 \pm 3.72	112.0 \pm 12.05
Insulin (μ U/mL)	16.9 \pm 1.11	18.7 \pm 2.18
HOMA-IR (μ U/mL \cdot mmol/22.5)	4.0 \pm 0.29	5.3 \pm 0.89
Serum cholesterol (mg/dL)	192.2 \pm 3.44	199.7 \pm 7.69
Serum LDL cholesterol (mg/dL)	116.8 \pm 1.39	121.5 \pm 4.19
Serum HDL cholesterol (mg/dL)	52.8 \pm 2.28	53.1 \pm 6.28
Serum non-HDL cholesterol (mg/dL)	139.5 \pm 3.58	146.6 \pm 3.74
Serum triglycerides (mg/dL)	143.1 \pm 14.71	160.7 \pm 19.05
Serum sitosterol (μ g/dL)	382.3 \pm 22.38	331.5 \pm 69.31
Serum campesterol (μ g/dL)	230.4 \pm 14.62	175.2 \pm 46.28
Serum lathosterol (μ g/dL)	179.8 \pm 8.23	248.4 \pm 34.31
7- α -OH-cholesterol (mg/h)	2.3 \pm 0.22	2.3 \pm 0.31
7- α -OH-cholesterol (μ g/dL)	5.6 \pm 0.31	5.5 \pm 0.82
27-OH-cholesterol (mg/h)	4.9 \pm 0.42	6.1 \pm 0.53
27-OH-cholesterol (μ g/dL)	11.3 \pm 1.65	13.9 \pm 1.73

Data presented as mean \pm SE or absolute numbers. No significant differences were found.

Statistical analysis

Data were expressed as means \pm SE. Inter-group comparisons and within-group differences were performed by Student *t* test for unpaired and paired data, respectively; changes before and after 6 months between the two surgical procedures were also compared by Student *t* test for unpaired data. All statistical analyses were performed by Statview software for Macintosh. $P < 0.05$ was considered statistically significant.

Sample size and power calculation

The sample size allowed for the detection of a 50% difference or more between subjects undergoing restrictive or malabsorptive procedures in total cholesterol decrease, with a type 1 error of 0.05 and a

power of 0.8. Assuming a difference in cholesterol decrease of 50 mg/dL with an SD of 10 mg/dL (2,5–7), and a rate of 1:1 between the two groups of subjects, 16 subjects were required. Assuming 15% would be noncompleters, 20 subjects were included.

RESULTS—Table 1 shows the clinical and metabolic data of obese subjects entering the study; all variables were similar in the two groups, with no significant difference.

Figure 1 shows that the decrease of BMI, HOMA-IR, and triglycerides was similar in the two groups; a small but significant decrease was observed in HDL cholesterol levels in subjects undergoing BIBP, but with no significant difference

from subjects undergoing AGB. Also, blood glucose and insulin levels decreased in a significant manner, with no difference between BIBP and AGB (not shown). In contrast, total cholesterol and LDL cholesterol levels were affected in a different way by BIBP and AGB, with a clear intergroup difference.

Figure 2 shows that non-HDL cholesterol, sitosterol, and campesterol levels decreased with BIBP, but not with AGB. Serum lathosterol increased with BIBP, but not with AGB, with a significant difference between BIBP and AGB.

Figure 3 shows that the rate of appearance and serum levels of 7- α -OH-cholesterol increased with BIBP, but not with AGB, with a significant difference between BIBP and AGB. The rate of appearance of 27-OH-cholesterol did not vary with either BIBP or AGB, but the trend was significantly different; the serum levels of 27-OH-cholesterol decreased in a similar way with BIBP and AGB.

CONCLUSIONS—The aim of this study was to assess intestinal cholesterol absorption, cholesterol synthesis, and cholesterol catabolism in obese subjects undergoing two different surgical techniques, one based on pure malabsorption (BIBP) and the other on restriction of the stomach (AGB). The studies were performed under basal conditions and 6 months later, when the decrease of BMI was expected to be similar. Although the decrease of blood glucose, insulin, HOMA-IR index, and triglycerides was similar between the two groups, significant differences between pre- and post-surgery cholesterol metabolism appeared in subjects undergoing BIBP, but not in subjects undergoing AGB. In particular, total cholesterol, LDL cholesterol, non-HDL cholesterol, campesterol, and sitosterol decreased, and lathosterol increased, together with the hepatic cholesterol catabolism pathway (7- α -OH-cholesterol).

This leads us to speculate that with malabsorptive surgery, cholesterol absorption decreases, which is associated with a clear decrease of LDL cholesterol (and of non-HDL cholesterol). As a compensation, cholesterol synthesis increases, and this is associated with enhanced hepatic catabolism. The increase in lathosterol, a marker of cholesterol synthesis, would also point to enhanced LDL receptor activity in the liver, which would lead to reduced circulating LDL levels. In contrast, cholesterol absorption is

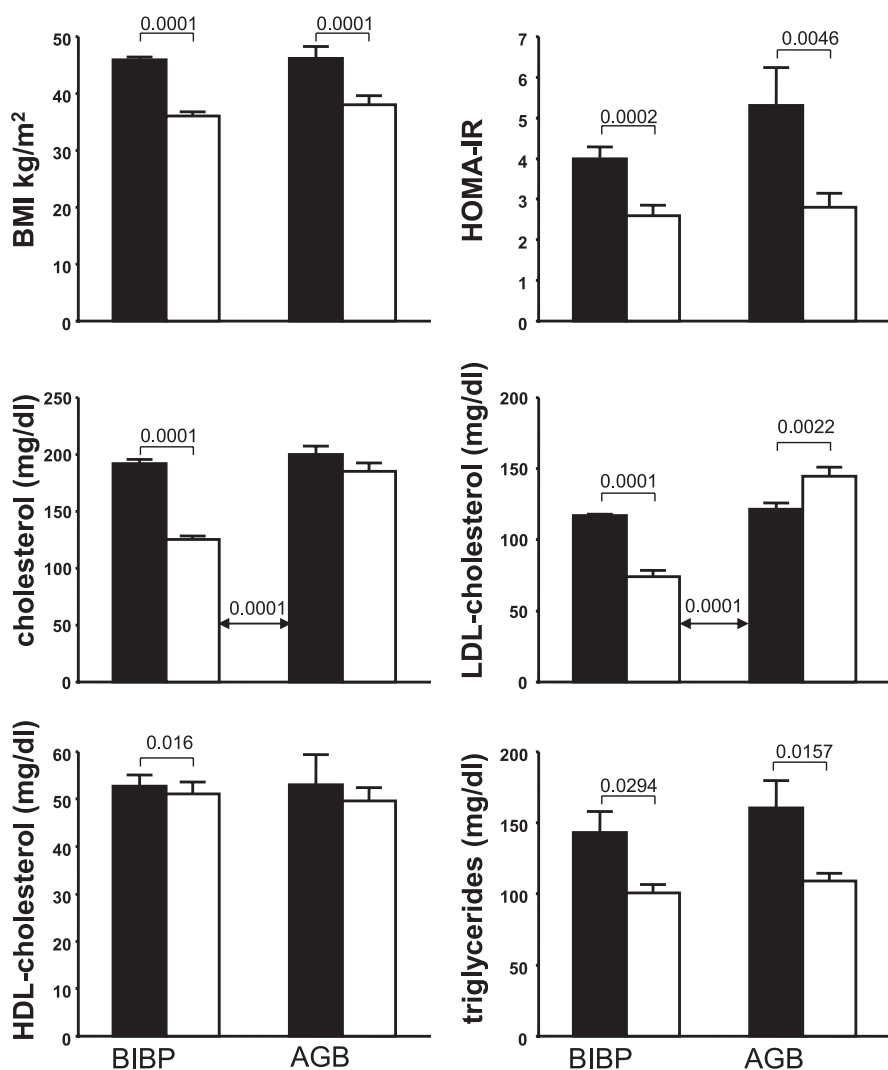


Figure 1—Metabolic changes induced by BIBP and AGB in grade 3 obesity. Baseline (black columns) and postsurgery (white columns) values are indicated. Means \pm SE. $n = 10$ in each panel. P values on top of columns indicate significance of pre- vs. postchange. P values between columns (two-sided arrows) indicate significance of intergroup differences.

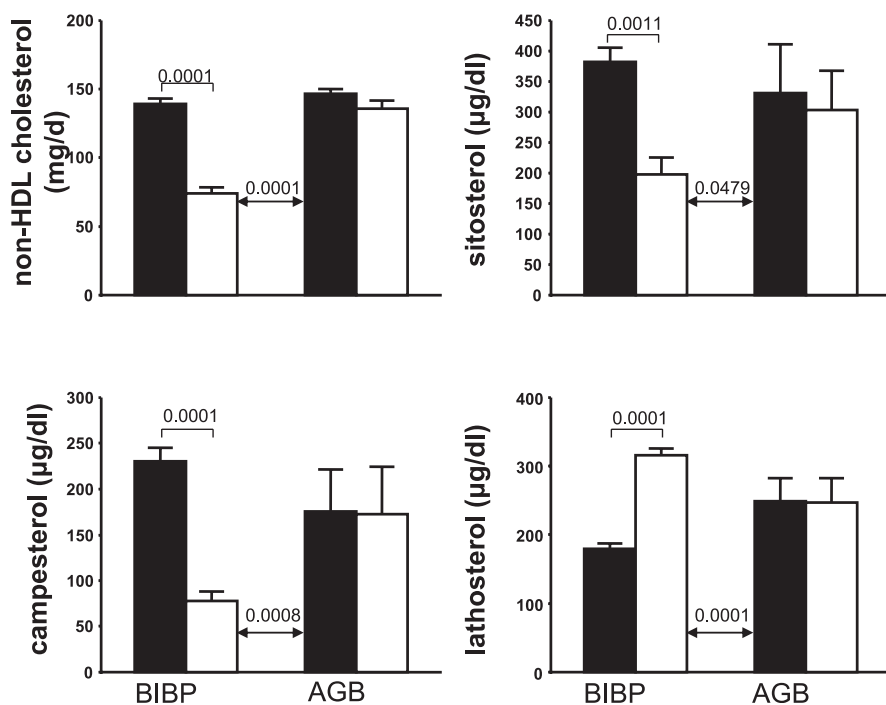


Figure 2—Changes of non-HDL cholesterol and of biomarkers of intestinal absorption of cholesterol (sitosterol and campesterol) and of cholesterol synthesis (lathosterol) induced by BIBP and AGB. Baseline (black columns) and postsurgery (white columns) values are indicated. Means \pm SE. $n = 10$ in each panel. P values between columns (two-sided arrows) indicate significance of intergroup differences.

unchanged in restrictive surgery, and LDL cholesterol shows a small but significant increase, if any. The only effect of purely restrictive surgery is a decrease in

extrahepatic cholesterol catabolism, parallel with an increase in LDL cholesterol, a finding that at present is unexplained. These data, obtained at 6 months, cannot

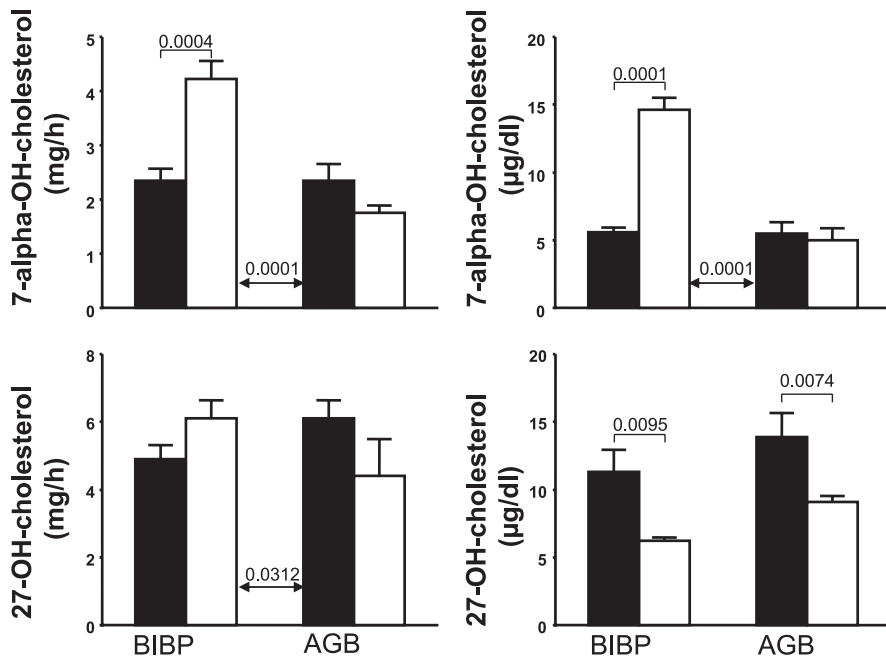


Figure 3—Rate of appearance (mg/h) and serum levels (µg/dL) of markers of bile acid metabolism before and after BIBP and AGB. Baseline (black columns) and postsurgery (white columns) values are indicated. Means \pm SE. $n = 10$ in each panel. P values between columns (two-sided arrows) indicate significance of intergroup differences.

be assumed as representative of the full picture of cholesterol metabolism afterward; in any case, these data indicate that different technical surgical techniques have a different effect on cholesterol levels, independent of weight loss.

Diet after surgery does not seem to be of importance, as differences between BIBP and AGB were negligible for the fat/cholesterol content; in addition, fat and cholesterol do not affect intestinal absorption of cholesterol as measured through plant sterols (10). However, it is noticeable that the different effects of BIBP and AGB persist for up to 3 or 6 years (2,5–7,18). The cholesterol reduction that we and others have reported after BIBP, BPD, or RYGB is quite a dramatic phenomenon and is likely due to the major reduction in cholesterol and bile acid reabsorption in the intestine, and possibly due to altered regulation of the feedback mechanisms controlled by nuclear proteins such as LXR, FXR, and peroxisome proliferator-activated receptor; these transcriptional factors are involved in bile acid and cholesterol metabolism in patients undergoing BIBP, BPD, or RYGB (which cause malabsorption and also reduce bile reabsorption) but not AGB (a purely restrictive bariatric procedure) (19). It is also possible that reduced gastric volume and reduced production of gastric lipase, as well as reduced secretion of cholecystokinin (which physiologically stimulates digestive enzyme secretion such as lipases and proteases), might result in a marked decrease in the hydrolysis of triacylglycerols, with a reduction of the absorption of free fatty acids (20). BPD and RYGB include partial gastric resection, or functional gastric disconnection; therefore, gastric bypass and BPD cannot be regarded as purely restrictive or purely malabsorptive surgical techniques. Blood glucose and serum insulin levels decreased to a similar extent with the two techniques, as was the case for the HOMA-IR index. These data indicate that changes of cholesterol absorption, synthesis, and catabolism were independent of glycemic control, insulin, and insulin resistance. In conclusion, compared with AGB, BIBP provides greater cholesterol lowering.

Acknowledgments—This study was supported by Grant 200825M9SC_002 (2008) from the Ministero dell'Istruzione dell'Università e della Ricerca (Rome, Italy).

No potential conflicts of interest relevant to this article were reported.

A.B. designed the study, performed in vivo studies, analyzed data, contributed to discussion, and wrote the paper. M.D.P. performed laboratory analysis and contributed to discussion. A.C. contributed to discussion. A.V. recruited patients and contributed to discussion. E.M., G.M., and V.P. performed bariatric surgery and contributed to discussion. F.F. instructed patients and contributed to discussion. A.E.P. designed the study, analyzed data, contributed to discussion, wrote the paper, and reviewed and edited the manuscript. A.E.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

- Buchwald H, Oien DM. Metabolic/bariatric surgery worldwide 2008. *Obes Surg* 2009;19:1605–1611
- Buchwald H, Estok R, Fahrback K, et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med* 2009;122:248–256, e5
- Doldi SB, Lattuada E, Zappa MA, Pieri G, Restelli A, Micheletto G. Biliointestinal bypass: another surgical option. *Obes Surg* 1998;8:566–569
- Buchwald H, Rudser KD, Williams SE, Michalek VN, Vagasky J, Connett JE. Overall mortality, incremental life expectancy, and cause of death at 25 years in the program on the surgical control of the hyperlipidemias. *Ann Surg* 2010;251:1034–1040
- Buchwald H, Avidor Y, Braunwald E, et al. Bariatric surgery: a systematic review and meta-analysis. *JAMA* 2004;292:1724–1737
- Pontirolì AE, Laneri M, Veronelli A, et al. Biliary pancreatic diversion and laparoscopic adjustable gastric banding in morbid obesity: their long-term effects on metabolic syndrome and on cardiovascular parameters. *Cardiovasc Diabetol* 2009;8:37
- Frige' F, Laneri M, Veronelli A, et al. Bariatric surgery in obesity: changes of glucose and lipid metabolism correlate with changes of fat mass. *Nutr Metab Cardiovasc Dis* 2009;19:198–204
- Pontirolì AE, Pizzocri P, Librenti MC, et al. Laparoscopic adjustable gastric banding for the treatment of morbid (grade 3) obesity and its metabolic complications: a three-year study. *J Clin Endocrinol Metab* 2002;87:3555–3561
- Del Puppo M, Crosignani A, Longo M, et al. A minimally invasive technique for the evaluation of the regulatory steps of the two major pathways of bile acid synthesis. *Clin Chim Acta* 2005;355:23–31
- Nissinen MJ, Gylling H, Miettinen TA. Effects of dietary cholesterol and fat on serum non-cholesterol sterols according to different apolipoprotein E subgroups among healthy men. *Br J Nutr* 2008;100:373–379
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–2497
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419
- Del Puppo M, Kienle MG, Petroni ML, Crosignani A, Podda M. Serum 27-hydroxycholesterol in patients with primary biliary cirrhosis suggests alteration of cholesterol catabolism to bile acids via the acidic pathway. *J Lipid Res* 1998;39:2477–2482
- Ciuffreda P, Casati S, Alessandrini L, Terraneo G, Santaniello E. Synthesis of deuterated isotopomers of 7 α - and (25R,S)-26-hydroxycholesterol, internal standards for in vivo determination of the two biosynthetic pathways of bile acids. *Steroids* 2003;68:733–738
- Javitt NB, Kok E, Lloyd J, Benschath A, Field FH. Cholest-5-ene-3 beta, 26-diol: synthesis and biomedical use of a deuterated compound. *Biomed Mass Spectrom* 1982;9:61–63
- Duane WC, Javitt NB. 27-hydroxycholesterol: production rates in normal human subjects. *J Lipid Res* 1999;40:1194–1199
- Corradini SG, Eramo A, Lubrano C, et al. Comparison of changes in lipid profile after bilio-intestinal bypass and gastric banding in patients with morbid obesity. *Obes Surg* 2005;15:367–377
- Repa JJ, Mangelsdorf DJ. Nuclear receptor regulation of cholesterol and bile acid metabolism. *Curr Opin Biotechnol* 1999;10:557–563
- Bays HE. Current and investigational anti-obesity agents and obesity therapeutic treatment targets. *Obes Res* 2004;12:1197–1211