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Reduced cardiovascular risk after bariatric surgery is linked to plasma ceramides, apolipoprotein-B100 and the ApoB100/A1 ratio

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Abstract

Background—Obesity-associated hyperlipidemia and hyperlipoproteinemia are risk factors for cardiovascular disease (CVD). Recently, ceramide-derived sphingolipids were identified as a novel independent CVD risk factor. We hypothesized that the beneficial effect of RYGB on CVD risk is related to ceramide-mediated improvement in lipoprotein profile.

Methods—A prospective study of patients undergoing RYGB was conducted. Patients clinical data and biochemical markers related to cardiovascular risk were documented. Plasma ceramide subspecies (C14:0, C16:0, C18:0, C18:1, C20:0, C24:0, and C24:1), ApoB100 and ApoA-1 were quantified preoperatively, 3 and 6 months post-RYGB, as was Framingham risk score. Brachial artery reactivity testing (BART) was performed before and 6 months after RYGB.

Results—Ten patients (9 female; age 48 yrs; BMI, 48.5±5.8 kg/m²) were included in the study. At 6 months post-op, mean BMI decreased to 35.7±5.0 corresponding to 51.3±10.0 % excess weight loss. Fasting total cholesterol, triglycerides, LDL, free fatty acids, ApoB100, ApoB100/ApoA-1 ratio and insulin resistance estimated from HOMA-IR were significantly reduced compared to pre-surgery values. The ratio of ApoB100/ApoA-1 correlated with a reduction in ceramide subspecies (C18:0, C18:1, C20, C24:0 and C24:1) (p<0.05). ApoB100 and the

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Disclosures/Conflicts of interest

Dr. Brethauer is a speaker, consultant, and scientific advisory board member for Ethicon Endo-Surgery, speaker for Covidien, and receives research support from Bard/Davol. Dr. Schauer's disclosures include: Ethicon Endo-Surgery: consultant, scientific advisory board member, research support; Remedy MD: board of directors; Stryker Endoscopy: scientific advisory board, educational grant; Bard/Davol: scientific advisory board, consultant; Gore: consultant, educational grant; Baxter: educational grant; Barosense, Surgique, Cardinal/Snowden Pencer: scientific advisory board; Covidien: educational grant; Allergan: educational grant; Surgical Excellence LLC: board of directors. Hazel Huang and Drs. Heneghan, Eldar, Gatmaitan, Kashyap, Kirwan, and Gornik have no conflicts of interest or financial ties to disclose.

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ApoB100/ApoA-1 ratio also positively correlated with the reduction in TG, LDL and HOMA-IR ($p<0.05$). BART inversely correlated with ApoB100 and total ceramide ($p=0.05$). Furthermore, the change in BART correlated with the decrease in C16:0 ($p<0.03$).

Conclusion—Our data suggests that improvements in lipid profiles and CVD risk factors after gastric bypass surgery may be linked to changes in ceramide lipid. Mechanistic studies are needed to determine if this link is causative or purely correlative.

Keywords

RYGB; bariatric surgery; cardiovascular risk; ceramide; sphingolipids; apolipoproteins; ApoB100; Apo A-1; ApoB100/Apo A-1 ratio

Introduction

Numerous risk factors for cardiovascular disease (CVD) have been identified, not least of which are obesity and its related comorbidities such as hyperlipidemia, hypertension, and diabetes mellitus¹. So strong are the associations between metabolic and cardiac diseases that the term ‘cardiometabolic syndrome’ became popular over the last decade, to describe the array of metabolic, hemodynamic and renal abnormalities which accompany visceral obesity and associated insulin resistance². The pathophysiological mechanisms underlying the cardiovascular sequelae of obesity are not completely understood. Proposed hypotheses include an increase in intra-abdominal pressure and the role of adipose tissue as an active endocrine and paracrine organ³. Adipocytes secrete numerous hormones, peptides and other molecules that affect metabolism, vascular function and glucose homeostasis⁴. An array of pro-inflammatory cytokines secreted by adipose tissue contribute to a state of low-grade inflammation, which possibly mediates the links between obesity, CVD and insulin resistance^{5, 6}.

Novel risk factors implicated in the cardiometabolic syndrome include lipoproteins and the sphingolipid ceramide species⁷. Sphingolipid, from which ceramides are derived, are major regulators of lipid metabolism; their synthesis has been shown to upregulate the expression of the sterol regulatory element-binding proteins (SREBPs) which directly activate at least 33 lipid-related genes⁸. Lipoproteins, a pseudomicellar structure comprised of a phospholipid and protein coat surrounding a core rich in cholesterol and triglycerides, transfer lipids from sites of production (e.g. liver and gut) to tissues where they are utilized for energy, membrane assembly, or hormone synthesis. Their fundamental protein components, apolipoproteins, are critical to the structure and function of lipoproteins – specifically for directing their metabolic fate⁹. ApoA-1 and ApoB100 are the structural proteins responsible for transporting high density lipoproteins (HDL) and the very low density lipoprotein (VLDL)-low density lipoprotein (LDL) spectrum, respectively. ApoA-1 lipoproteins mediate reverse cholesterol transport, transferring excess cholesterol from peripheral tissues back to the liver where it can be excreted in bile. ApoB100 containing lipoproteins transport VLDL and remnant particles from liver and gut generally, to sites of utilization.

Elevated plasma ceramides and apolipoproteins are implicated in the development of atherosclerotic CVD through several mechanisms¹⁰. In addition to their involvement in the transcriptional regulation of lipogenesis, increased ceramide and sphingomyelin (SM) content in oxidized remnant lipoproteins enhances their susceptibility for aggregation and retention in arterial walls. The ceramide metabolite, SM, reduces clearance of remnant lipoproteins by blocking access to lipoprotein lipase and Apolipoprotein E (ApoE), a ligand for LDL receptor binding¹¹. Inhibition of ceramide biosynthesis by myriocin, in an ApoE knockout murine model of atherosclerosis, significantly reduced atherosclerosis and

improved hepatic and plasma lipids and lipoprotein levels¹². ApoA-1 levels have been inversely related to the risk of cardiac events such as myocardial infarction¹³. The ApoB100/ApoA-1 ratio is one of the strongest novel risk factors for coronary heart disease and may be more informative than actual levels of either lipoprotein alone^{13, 14}. Abnormal levels of adipose tissue and/or plasma ceramides, ApoB100 and the ApoB100/ApoA-1 ratio have also been reported in obese individuals, and correlate with the degree of insulin resistance and inflammation^{15, 16}.

Bariatric surgery is the most effective treatment for obesity and its associated state of metabolic disarray. The Roux-en-Y gastric bypass (RYGB) is the most commonly performed and successful bariatric procedure in the US at present. In addition to substantial weight loss, RYGB leads to dramatic improvements in glycemic control, insulin sensitivity, and CVD risk¹⁷. However, the mechanisms underlying such effects have not been fully elucidated. We recently reported that improvements in the proinflammatory state and insulin sensitivity seen after gastric bypass surgery may be partly mediated by changes in ceramide levels¹⁸. We now hypothesize that the beneficial effect of RYGB on cardiovascular risk is related to a ceramide-mediated improvement in lipoprotein profile, specifically to changes in the ApoB100/ApoA-1 ratio. The primary aim of this study was to serially quantify apolipoproteins (ApoA-1 and ApoB100) and individual ceramide subspecies in the circulation of severely obese patients undergoing RYGB. We examined the relationship between apolipoproteins, the ApoB100/ApoA-1 ratio, and plasma ceramide levels, with clinical and laboratory-based indicators of cardiovascular risk pre- and postoperatively.

Materials and methods

Study cohort

This prospective longitudinal study was approved by the Institutional Review Board, and all patients gave written informed consent. Our study cohort consisted of 10 severely obese patients undergoing laparoscopic RYGB, on the basis that they met the criteria for bariatric surgery as outlined by the National Institutes of Health Consensus Development Panel report of 1991¹⁹. Individuals with known autoimmune disease, cancer, thrombotic disorders, and valvular heart disease were excluded, as were those who were unable or unwilling to cooperate with post-operative follow-up. Consenting participants were evaluated at three time-points; preoperatively, 3 and 6 months post-RYGB. Each evaluation comprised a clinical and physical review, anthropometric measurements, cardiovascular risk assessment, and blood sampling for measurement of biochemical, metabolic, and inflammatory biomarkers. Blood samples were collected after a 12-hour fast using evacuated blood collection tubes containing EDTA. Laparoscopic RYGB was performed as described previously²⁰

Analytical Measurements

Fasting blood samples were analyzed for concentrations of the following biochemical markers: fasting plasma glucose, insulin, free fatty acids, triglycerides (TG), total cholesterol, HDL, LDL, ceramide subspecies (C14:0, C16:0, C18:0, C18:1, C20:0, C24:0, C24:1) and the apolipoproteins ApoA-1 and ApoB100. Ceramide species were quantified in prepared plasma samples by HPLC on-line electrospray ionization tandem mass spectrometry, as previously described²¹. Ceramide subspecies were quantified (nmol/ml) using calibration curves and the ratios of the integrated peak areas of ceramide subspecies and internal standards. Total measured ceramide was calculated from the sum of C14:0, C16:0, C18:1, C18:0, C20:0, C24:0 and C24:1 ceramide subspecies. Plasma levels of ApoB100 and ApoA-1 were measured using the human apolipoprotein assay kit (#APO-62

K) and protocol available from Millipore (Billerica, MA), on a MILLIPLEX® MAP platform, according to the vendor's guidelines.

Clinical measurements

Vascular reactivity was measured as a surrogate marker for endothelial function, by means of a brachial artery reactivity test at baseline and at the 6-month postoperative assessment²². In this assessment, a normal response of the brachial artery to reactive hyperemia is 10–15% vasodilation; impaired relaxation (<10%) suggests subclinical atherosclerotic disease²³. Insulin resistance was estimated using the previously validated homeostasis model assessment of insulin resistance²⁴. Cardiovascular risk was determined at baseline and at both time points post-operatively using the gender specific Framingham Coronary Heart Disease risk score developed by Wilson *et al*²⁵.

Statistical analysis

All statistical analyses were performed using PASW Statistics programme version 18 (SAS Institute, Cary, NC, USA). Data are presented as mean ± SD. Comparisons over time for the apolipoproteins, ceramide species, and key outcome variables was performed using repeated measures analysis of variance (ANOVA), and associations between variables were determined using Pearson's correlation analyses. In all tests, $p < 0.05$ was considered statistically significant.

Results

Patient characteristics and surgical outcome

Ten patients (9 female, mean age for the group 48.6 ± 9.6 yrs and preoperative BMI 48.5 ± 5.8 kg/m²) were included in this study. Baseline demographics and clinical characteristics are outlined in Table 1. All patients had at least one obesity-related comorbidity, including hypertension, hyperlipidemia, diabetes mellitus and obstructive sleep apnea. Postoperative weight loss is also documented in Table 1; body weight and BMI had decreased significantly by 6 months postoperatively. The percent of excess weight loss (%EWL) at 3 and 6 months was $38.2 \pm 6.8\%$ and $51.3 \pm 10.0\%$, respectively. Of three patients with diabetes mellitus, two experienced remission within the first 3 months postoperatively, as defined by the American Diabetes Association's criteria of normal glycemic measures with no active pharmacologic therapy²⁶. The other diabetic patient had an improvement in glycemic indices and reduction in oral hypoglycemic requirements at 3 and 6 months post-RYGB.

Changes in metabolic indices, insulin resistance and cardiovascular risk post-RYGB

We have previously reported significant decreases in a variety of metabolic indices and proinflammatory markers after gastric bypass, observed in a larger cohort of patients inclusive of the ten current study participants¹⁷. Specific results for the present group of patients are illustrated in Table 2. Herein, we again observed significant decreases in levels of fasting plasma insulin, free fatty acids, total cholesterol, low-density cholesterol, and HOMA-IR at both 3 and 6 months postoperatively. The mean % increase in brachial artery reactivity seen in these ten patients at 6 months was $4.2 \pm 10.1\%$. Although this change did not meet statistical significance ($p = 0.17$), it is suggestive of an improvement in endothelial function. Ten-year cardiovascular risk, as measured by the FRS, decreased with surgical-induced weight loss. At 3 months the FRS had decreased by 33.5% and at 6 months by 43.6%, however these decreases were not statistically significant (Figure 1).

We also recently reported that plasma ceramide levels decrease post-RYGB, and that the decrease in total ceramide levels correlate with increased insulin sensitivity¹⁸. Additionally, in this study we observed a significant decrease in levels of ApoB100 lipoprotein (average

decreases of 22.9% at 3 months and 32.1% at 6 months, $p=0.01$ and $p=0.02$, respectively) and a corresponding decrease in the ApoB100/ApoA-1 ratio (average decreases of 4.5% at 3 months and 22.5% at 6 months, $p=0.27$ and $p=0.04$, respectively), both of which are considered novel cardiovascular risk factors (Table 2, Figure 2).

Correlation of apolipoprotein changes with ceramide levels and cardiovascular risk postoperatively

At 3 months post-RYGB, the ApoB100/ApoA-1 ratio correlated significantly with levels of total ceramides and C18:0, C18:1, C20:0, C24:0 ceramide subspecies (Pearson's correlation coefficients illustrated in Table 3). At 6 months, there remained a trend toward decreasing ceramide levels in association with a continued decrease in the ApoB100/ApoA-1 ratio although the correlations were no longer significant at this time point. The percent change in ApoB100 and in the ApoB100/ApoA-1 ratio at both time points also showed interesting correlations with ceramide subspecies, and lipid levels. At 3 months, the % change in the ApoB100/ApoA-1 ratio correlated with the % change in C18:1 ($r=0.68$, $p=0.03$), while the correlation with changes in total ceramide levels neared significance ($r=0.56$, $p=0.09$). The postoperative change in ApoB100/ApoA-1 ratio also correlated significantly with the changes in total cholesterol, LDL, and triglyceride levels ($p=0.01$, $p=0.01$, and $p=0.002$, respectively). Furthermore, the change in this ratio correlated negatively with the % EWL ($r=-0.62$, $p=0.05$) reflecting the fact that the greater the weight loss postoperatively, the greater the decrease in ApoB100/ApoA-1 ratio. At 6 months, the change in the apolipoprotein ratio correlated with the % change in C14:0 ($r=0.69$, $p=0.03$), and the % change in ApoB100 alone correlated with the change in triglyceride levels.

Changes in the apolipoprotein ratio and ceramide levels after surgery were observed to correlate with endothelial reactivity, and with clinical indicators of cardiovascular risk and insulin sensitivity. At 6 months post-RYGB, the decrease in the ApoB100/ApoA-1 ratio correlated inversely with brachial artery reactivity ($r=-0.72$, $p=0.02$). The % change in FRS was closely associated with the % change in total ceramides ($r=0.67$, $p=0.04$), C14:0 ($r=0.63$, $p=0.07$), C18:1 ($r=0.63$, $p=0.07$), and C24:1 ($r=0.66$, $p=0.05$). Finally, the % change in total ceramides correlated with the % change in HOMA-IR ($r=0.71$, $p=0.02$), and with an improvement in BART ($r=-0.74$, $p=0.03$).

Discussion

This prospective study, examining the change in novel cardiovascular biomarkers in a bariatric patient population, is the first to explore the potential role of apolipoproteins and ceramides in mediating the cardiometabolic benefits of weight loss surgery. Building on previous reports from our research group which demonstrated that bariatric surgery induces significant reductions in plasma ceramides, pro-inflammatory markers, and other indicators of CVD¹⁷¹⁸, we now present preliminary data demonstrating that apolipoproteins are also significantly altered post-RYGB. We believe the changes in ApoB100 and the ApoB100/ApoA-1 ratio are likely to be mediated by the simultaneous decrease in ceramide production, given the significant correlations observed between these lipid moieties as well as with other risk factors for CVD. In addition to observing decreases in absolute values of ApoB100, the ApoB100/ApoA-1 ratio, and ceramides, the fact that we observed significant correlations between the percent changes in both apolipoproteins and ceramide levels strengthens the case that the intervention (RYGB) was responsible for alterations in these parameters. Baseline physiology levels often vary widely between individuals, particularly among obese individuals with states of metabolic disarray. Using the percentage change in parameters from baseline can therefore be a more robust method of analysis. We also observed that the postoperative percent change in the ApoB100/ApoA-1 ratio correlated with the percent change in total cholesterol, LDL, and TG levels, and with the improvement

in patient's endothelial reactivity as measured by BART. These interesting associations lead us to believe that gastric bypass was primarily responsible for reducing the risk of atherosclerotic CVD, as indicated by alterations in these risk factors, and that apolipoproteins and the ceramide sphingolipids could be the potential mediators' of these effects.

It is well known that weight loss induces favorable changes in cholesterol levels, blood pressure, glycemic indices and weight-related diseases. Such effects are particularly evident following the dramatic weight loss that occurs after bariatric procedures such as RYGB. Accumulating evidence also suggests that surgically-induced weight loss is associated with reduced cardiovascular mortality, and overall mortality²⁷. However, the precise mechanisms underlying the reduction in CVD risk following weight loss are incompletely understood. Postulated mechanisms of cardiovascular protection include a decrease in inflammation, decreased intra-abdominal pressure as a result of reduced visceral fat, and improved endothelial function^{28, 29}. We hypothesized that changes in lipid metabolism also play a part in effecting the cardiovascular benefits of weight loss and bariatric surgery. Kullberg *et al* have previously demonstrated that weight loss resulting from a low-calorie diet or gastric bypass surgery resulted in significant lipid mobilization as soon as one month after intervention; the magnitude of these effects was greater among surgical patients despite equivalent weight loss³⁰. Caloric restriction and loss of adipose tissue mass has been shown to cause decreases in hepatic lipogenesis, hepatic VLDL-TG secretion, hepatic fat content, gene expression of hepatic chemokines, and hepatic fibrogenesis³¹. It has also been shown previously that apolipoprotein secretion changes with weight loss, although data in this field is less consistent³². Cholesterol levels have a direct effect on regulation of lipoprotein levels; individuals with hypercholesterolemia for example have increased Apo-B containing lipoproteins consequent in part to decreased clearance of particles by receptors⁹. It is not surprising then that interventions such as bariatric surgery, which induce changes in cholesterol levels through weight loss or other mechanisms, would also affect lipoprotein concentrations. It is well reported that changes in an individual's lipid profile affects CVD risk, and indeed mortality³³. However, this is the first time that an association has been identified between post-bariatric surgery changes in apolipoprotein levels, ceramide sphingolipids, and reduction in CVD risk.

Several findings from this and other reports³⁰ suggest that improvements in lipid metabolism after gastric bypass surgery may be weight-independent effects of the surgery, specifically due to its malabsorptive component. Evidence to support this includes the fact that changes observed in lipids, apolipoproteins, and ceramide levels occur early after surgery, even before significant weight loss occurs. Furthermore, patients with non-surgical induced malabsorptive diseases are known to have abnormal lipid metabolism and low plasma cholesterol³⁴. Interruption of the entero-hepatic cycle of bile acids and a decrease in the absorption of cholesterol result in enhanced bile acid synthesis from cholesterol, which stimulates uptake of cholesterol rich lipoproteins by the liver³⁴. These effects may partly explain the changes we observed in circulating apolipoprotein and ceramide levels post-RYGB.

The regulation of lipid metabolism by sphingolipids is known to occur through their regulation of genes that synthesize key proteins of cholesterol, phospholipid and fatty acid metabolism³⁵. Worgall *et al* have also demonstrated that ceramides correlate with increased transcription and expression of genes and proteins that mediate cholesterol metabolism, including the SREBP family of transcriptional factors. Although there is no direct evidence to date linking ceramide and lipoprotein levels, it is intuitive that as ceramides affect cholesterol levels at the transcriptional stage, then lipoprotein levels would also reflect properties of ceramide and sphingolipid metabolism. Sphingolipids have been indicated in

the pathogenesis of a variety of metabolic diseases, including obesity and its associated states of insulin resistance and atherosclerosis¹⁶. In fact sphingolipid metabolism, of which ceramides are a product, is activated by the constellation of proinflammatory markers and adipokines secreted from visceral fat¹⁶. Ceramide signaling has also been shown to induce particular inflammatory responses in atherosclerotic lesions, such as smooth muscle cell proliferation. Additionally, these sphingolipid metabolites have been shown to contribute to the instability and rupture of atherosclerotic plaques¹⁶. Decreasing the activity of sphingolipid and ceramide pathways, through weight loss or weight independent effects of gastric bypass surgery, may therefore be an important mechanism in CVD risk reduction. Data from this study, such as the inverse correlation observed between the ApoB100/ApoA-1 ratio and brachial artery reactivity at 6 months postoperatively, as well as the strong correlations between the percent change in total ceramide levels and the improvement in brachial artery reactivity and Framingham risk scores after gastric bypass, supports this hypothesis.

This pilot study had some limitations, particularly the small subject numbers. It is unlikely to be powered sufficiently to detect the relevance of much smaller changes in lipoprotein and ceramide levels on CVD risk. Larger prospective studies are necessary to further extrapolate the magnitude of the effect of sphingolipid metabolism and lipoprotein levels on atherosclerotic disease, and the extent of the effect of bariatric surgery on altering that relationship. Further, a correlation between variables does not prove causality; additional mechanistic studies are needed to determine if this link is indeed causative or purely associative.

Conclusion

This study is the first to show a relationship between altered ceramide levels after bariatric surgery in morbidly obese patients, and decreased risk factors for CVD such as lipoprotein and lipid levels, brachial artery reactivity, and the Framingham risk score for heart disease. The improvements observed in cardiovascular risk parameters as soon as 3–6 months after gastric bypass further highlight the major benefits of bariatric surgery, and support the development and application of surgical approaches for the treatment of obesity and for CVD risk reduction.

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Figure 1. Framingham risk score (FRS) at baseline (preoperatively), 3 months and 6 months post RYGB.

Figure 2.
Change in levels of apolipoprotein (Apo) B100 (a) and in the ApoB100/ApoA-1 ratio (b) at 3 and 6 months after RYGB.

Table 1Patient characteristics and surgical outcomes [Mean $\pm$ SD]

	Pre-op	3 months post-RYGB	6 months post-RYGB	P value *
N	10	10	10	-
Age, years	48.6 $\pm$ 9.6	-	-	-
Gender %M/F	10/90	-	-	-
Comorbidities				
Diabetes Mellitus	30%	10%	10%	0.107
Hypertension	90%	50%	50%	0.292
Hyperlipidemia	80%	50%	40%	0.197
Obstructive Sleep	30%	10%	0%	-
Weight (lb)	294.2 $\pm$ 61.4	237.8 $\pm$ 55.8	217.7 $\pm$ 52.1	0.015
BMI (kg/m²)	48.5 $\pm$ 5.8	38.8 $\pm$ 5.0 [^]	35.7 $\pm$ 5.0	<0.001

* p-values represent the significance of the difference between preoperative and 6 month post-RYGB values.

[^] The difference between preoperative and 3 month post-RYGB values is significant

BMI, Body mass index

Table 2Metabolic markers, endothelial reactivity and Framingham risk score pre- and postoperatively [Mean $\pm$ SD]

N	Pre-op	3 months post- RYGB	6 months post- RYGB	P value *
	10	10	10	
HbA1c (%)	6.0 $\pm$ 1.5	5.5 $\pm$ 1.0	5.4 $\pm$ 0.9	0.33
FPG (mg/dl)	103.4 $\pm$ 42.0	91.9 $\pm$ 32.9	85.4 $\pm$ 19.4	0.24
FPI (pg/ml)	630.9 $\pm$ 389.5	338.2 $\pm$ 207.5 ^	302.7 $\pm$ 118.7	0.03
HOMA-IR	2.9 $\pm$ 1.6	1.3 $\pm$ 0.7 ^	1.1 $\pm$ 0.6	0.01
FFA (mmol/l)	0.6 $\pm$ 0.2	0.4 $\pm$ 0.2 ^	0.3 $\pm$ 0.2	<0.01
Total cholesterol	209.6 $\pm$ 38.0	172.9 $\pm$ 35.9 ^	173.7 $\pm$ 41.8	0.06
LDL (mg/dl)	130.5 $\pm$ 33.7	102.3 $\pm$ 25.9 ^	94.9 $\pm$ 28.0	0.02
HDL (mg/dl)	50.0 $\pm$ 18.0	47.7 $\pm$ 14.4	59.2 $\pm$ 23.1	0.34
Tg (mg/dl)	144.9 $\pm$ 53.1	114.8 $\pm$ 39.4	115.1 $\pm$ 49.6	0.21
Total ceramides	8.8 $\pm$ 2.3	7.1 $\pm$ 1.6	7.1 $\pm$ 1.0	0.05
C14:0	0.13 $\pm$ 0.06	0.10 $\pm$ 0.04	0.10 $\pm$ 0.04	0.25
C16:0	1.8 $\pm$ 0.4	1.5 $\pm$ 0.2	1.5 $\pm$ 0.3	0.06
C18:0	0.8 $\pm$ 0.2	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.04
C18:1	0.15 $\pm$ 0.07	0.13 $\pm$ 0.03	0.11 $\pm$ 0.03	0.13
C20:0	0.38 $\pm$ 0.12	0.30 $\pm$ 0.08	0.30 $\pm$ 0.08	0.07
C24:0	4.2 $\pm$ 1.8	3.1 $\pm$ 1.1	3.3 $\pm$ 1.0	0.21
C24:1	1.3 $\pm$ 0.6	1.4 $\pm$ 0.5	1.2 $\pm$ 0.3	0.67
ApoA-1 ($\times 10^6$, ng/mL)	4.0 $\pm$ 0.8	3.5 $\pm$ 0.9	3.8 $\pm$ 1.1	0.56
ApoB100 ($\times 10^6$, ng/mL)	3.6 $\pm$ 1.0	2.7 $\pm$ 0.6 ^	2.4 $\pm$ 0.8	0.01
ApoB100/ApoA-1 ratio	0.92 $\pm$ 0.3	0.78 $\pm$ 0.2	0.66 $\pm$ 0.2	0.04
BART	20.1 $\pm$ 6.9 %		24.3 $\pm$ 6.3 %	0.17
Framingham risk	5.0 $\pm$ 4.4 %	2.8 $\pm$ 3.1 %	2.3 $\pm$ 2.2 %	0.13

* p-values represent the significance of the difference between preoperative and 6 month post-RYGB values.

^ The difference between preoperative and 3 month post-RYGB values is also significant

FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, Homeostatic model of assessment of insulin resistance; TG, triglycerides; FFA, fasting plasma free fatty acids; BART, Brachial artery reactivity testing; FRS, Framingham risk score (10 year risk)

Table 3

Correlation of ApoB100/ApoA-1 ratio with plasma ceramide levels at 3 and 6 months post-RYGB

Correlation with ApoB100/ApoA-1 ratio:		Total ceramides	C14:0	C16:0	C18:0	C18:1	C20:0	C24:0	C24:1
<u>3 months post-RYGB</u>									
Pearson's correlation		0.86	-0.33	0.54	0.63	0.69	0.71	0.67	0.60
coefficient p-value		<0.01	0.35	0.11	0.05	0.03	0.02	0.03	0.07
<u>6 months post-RYGB</u>									
Pearson's correlation		0.48	-0.03	0.23	0.48	0.04	0.15	0.35	-0.04
coefficient p-value		0.16	0.94	0.52	0.16	0.90	0.68	0.33	0.91