Effects of bariatric surgery on HDL structure and functionality: results from a prospective trial

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KEYWORDS: High-density lipoprotein; Structure; Function; Morbid obesity; Bariatric surgery

BACKGROUND: In addition to high-density lipoprotein cholesterol (HDL-C) levels, HDL quality appears also very important for atheroprotection. Obese patients with metabolic syndrome have significantly reduced HDL-C levels and are usually at increased risk for coronary heart disease. Despite that weight loss benefits these patients, its effects on HDL quality and functionality is currently poorly studied.

OBJECTIVES: We investigated how rapid weight loss affects HDL structure and its antioxidant potential in patients undergoing a malabsorptive bariatric procedure.

METHODS: Fasting plasma samples were collected the day before and 6 months after the bariatric procedure from 20 morbidly obese patients with body mass index >50, then HDL was isolated and analyzed by biochemical techniques.

RESULTS: We report a dramatic alteration in the apolipoprotein ratio of HDL that was accompanied by the presence of more mature HDL subspecies and a concomitant increase in the antioxidant potential of HDL. Interestingly, our obese cohort could be distinguished into 2 subgroups. In 35% of patients (n = 7), HDL before surgery had barely detectable apolipoprotein (apo) A-I and apoCIII, and the vast majority of their HDL cholesterol was packed in apoE-containing HDL particles. In the remaining 65% of patients (n = 13), HDL before surgery contained high levels of apoA-I and apoCIII, in addition to apoE. In both subgroups, surgical weight loss resulted in a switch from apoE to apoA-I–containing HDL.

CONCLUSIONS: Rapid weight loss exerts a significant improvement in HDL structure and functionality that may contribute to the documented beneficial effect of malabsorptive bariatric procedures on cardiovascular health.

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Introduction

The inverse correlation between high levels of plasma high-density lipoprotein cholesterol (HDL-C) and coronary heart disease morbidity and mortality seen in numerous epidemiological studies, coupled with a number of atheroprotective properties of HDL, identified HDL-C levels as a novel pharmacological target for the prevention and treatment of coronary heart disease. Based on this epidemiological correlation, it was proposed that increasing HDL-C levels by pharmacological means may lead to a reduction of coronary heart disease (CHD)-related mortality. However, the recent failure of 2 cholesteryl ester transfer protein (CETP) inhibitors in clinical trials as well as mendelian randomization studies of genetic mutations that influence HDL-C have questioned HDL-C as a surrogate measure for coronary risk and have reinforced the concept of “HDL quality.” This concept refers to the composition and functions of HDL present in a single individual that may define its atheroprotective or proatherogenic potential. To date, however, HDL quality still remains poorly defined.

It is well-established that HDL particles are heterogeneous in size and apolipoprotein composition. In support of HDL's role in the prevention of coronary heart disease, surgical procedures have been shown to be effective for the treatment of morbid obesity. Body weight and adipose tissue mass reductions after this operation have been associated with improvement in plasma cholesterol profile, although its effects on HDL particle composition and functionality have not been investigated thus far.

To identify measurable markers of HDL “quality” that may replace HDL-C as a surrogate measure of the risk for myocardial infarction, we turned out attention to the relevant clinical example of rapid weight loss after bariatric surgery. Patients who undergo rapid weight loss after malabsorptive procedures enjoy a reduced risk of CHD later on in life, mainly because of the improvement in their plasma lipid and lipoprotein profile. In the present prospective observational study, we focused on patients undergoing a variant of biliopancreatic diversion, namely biliopancreatic diversion with Roux en Y gastric bypass reconstruction and with long limbs (BPD-RYGB-LL). In particular, we isolated HDL from plasma of morbidly obese patients with a BMI of 50 or higher the day before and 6 months after surgery and then performed biochemical and structural analyses.

Methods

Patient characteristics

Twenty superobese patients (BMI ≥50) who were scheduled to undergo BPD-RYGB-LL (preferred malabsorptive bariatric procedure for superobese patients) and were admitted to the Department of Surgery-Morbid Obesity Unit of the University Hospital of Patras between September 2011 and January 2012 were recruited. A 6-month follow-up was performed to the same group of patients to ensure patients’ recovery and outcomes. Seven healthy, lean patients (BMI <27) who were admitted to the same hospital to undergo laparoscopic cholecystectomy served as the control (C) group. All study participants were free of diabetes, dyslipidemia, or hypertension. As a result, they received no medication for these disorders, and no hormonal supplementation. These strict selection/exclusion criteria reduced significantly the number of participants enrolled in the study; however, it allowed for the precise evaluation of the role of rapid weight loss on HDL structure and antioxidant function in the absence of any comorbidities that could affect these parameters. Obese patients in both the 35% subgroup and 65% subgroup were selected to be clinically and demographically identical for the purpose of bariatric surgery. The study conforms with the principles outlined in the Declaration of Helsinki and was approved by the ethics committee of the University Hospital of Patras. All subjects gave their informed consent.

Surgical technique

The malabsorptive bariatric procedure used in this study, was BPD-RYGB-LL. In all cases, the procedure was performed by laparotomy.

Blood sampling and plasma lipid determination

Peripheral blood samples were collected after overnight fasting for assessing plasma lipid levels. Plasma triglycerides, total cholesterol, and free cholesterol were determined spectrophotometrically, as described previously.
Fractionation of plasma lipoproteins by density gradient ultracentrifugation

Four hundred microliters of plasma from each individual as well as 400 μL of pools of plasma from each of the 3 subject groups were fractionated by density gradient ultracentrifugation (UCF), as described previously.11

Western blot analysis

Western blot analysis for apoA-I, apoE, apoCIII, apoCI, and paraoxonase 1 (PON1) was performed as described previously11 using the following primary antibodies: a goat anti-human apoA-I antibody (cat #K45252 G), a goat anti-human apoE antibody (cat #K74190 G), and a goat anti-human apoCIII antibody (cat #K74140 G; all Biodesign International, ME, USA); rabbit anti-human apoCI (cat # 31A-R1a, Academy Biomedical Company, TX, USA); and a goat anti-human PON1 antibody (cat # AP16947PU-N, Acris antibodies, CA, USA). Semiquantitative determination of relative apolipoproteins A-I, E, CIII, and CI levels was performed by ImageJ free software (http://rsbweb.nih.gov/ij/).

Oxidation assay

For the analysis of the oxidation potential of HDL we used the dihydrorhodamine 123 method of Kelesidis et al.22 HDL oxidative function was calculated as the mean of quadruplicates for each well.

Electron microscopy analysis of HDL particles

HDL fractions were visualized by electron microscopy, then photographed and analyzed as described previously.23 Briefly, after application of upper limits for the size of HDL particles (<15 nm), 356 HDL particles from the C group, 560 HDL particles from the preoperative (pre) group, and 356 HDL particles from the postoperative (post) group entered the statistical analysis. By visual inspection, the distributions of HDL particle diameter and area for the samples were evidently skewed. The deviation from normality was confirmed using the Kolmogorov-Smirnov and the Shapiro-Wilk tests, both showing that our measurements should be treated with non-parametric tests (P < .001). Image analysis of electron microscopy (EM) pictures was followed by Kruskal-Wallis nonparametric statistical analysis for the comparison of the diameter, surface area, and particle distribution of the HDL between the pre and post samples.

Nondenaturing 2-dimensional electrophoresis analysis of HDL fractions

The analysis of HDL subpopulations in the plasma of patients preoperatively and 6 months postoperatively by nondenaturing 2-dimensional electrophoresis was performed as described previously.24 The assignment of mobility to the various HDL subpopulations was based on the work of Asztalos and Schaefer.25

Lecithin-cholesterol acyltransferase activity assay

Total plasma, from all patients preoperatively and 6 months postoperatively as well as from control subjects, was used as the source of lecithin:cholesterol acyl transferase (LCAT) enzyme. The reconstituted HDL particles used in the assay were prepared by the sodium cholate dialysis method as described previously.26 The enzymatic reactions were carried out as described and the apparent maximum reaction velocity (Vmax) and Michaelis constant (Km) were derived as described previously.27

Cholesteryl-ester transfer protein activity assay

The activity of CETP in the plasma was performed using the CETP activity assay kit (cat# KA0790, Abnova, Taipei City, Taiwan), according to the manufacturer’s instructions. The activity was determined as picomoles of cholesteryl esters per microliter of plasma per hour ± standard error of the mean. Statistical analysis was performed by analysis of variance.

Statistical analysis

For the comparison of data between any 2 groups, statistical analysis was performed using the Student t-test. Where more than a 2-group comparison was required (CETP and LCAT activities) the results were analyzed using analysis of variance. For the analysis of the electron microscope data the deviation from normality was confirmed using the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Image analysis of EM pictures was followed by Kruskal-Wallis, whereas specific comparisons between HDL particle subpopulations were performed using the z-test. Data are reported as mean ± standard error of the mean. A P value <.05 was considered significant. In figures 3, 4, and 5 * indicates P < .05 and ** indicates P < .005. All analyses comparing properties of HDL between the preoperatively and postoperatively states were paired (apolipoprotein ratios, antioxidant potential, and plasma cholesterol levels). Independent measurements were made when comparing the C group with the pre and post states.

Results

Clinical characteristics and lipid levels

In this study, we analyzed 3 different groups of plasma samples: the C group isolated from normal-BMI patients admitted to the hospital for laparoscopic cholecystectomy, the pre group isolated from superobese patients the day before BPD-RYGB-LL, and the post group isolated from
these same patients 6 months after the operation (6-month follow-up samples). The selection of study participants is described in the Methods. The clinical characteristics and lipid levels of pre and post groups are shown in Table 1.

**Apolipoprotein and PON1 composition of lipoprotein fractions**

After UCF separation of lipoproteins, the distribution of apoA-I, apoE, apoCI, apoCIII, and PON1 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blotting. This analysis revealed an impressive change in apolipoprotein content of HDL after rapid weight loss, 6 months after surgery. Specifically, we observed that operated patients could be further divided into 2 subgroups. In 35% of them (n = 7), HDL before surgery had barely detectable apoA-I and apoCIII, and the vast majority of their HDL-C was packed in apoE-containing HDL particles (Fig. 1A). After surgery, apoA-I levels were restored, whereas only trace amounts of apoCIII and apoE were present in HDL (Fig. 1B). In the remaining 65% of patients (n = 13), in the pre samples, we discovered high levels of apoA-I, apoCIII, and apoE in all HDL fractions (Fig. 2A). Six months after surgery the HDL-C of these patients was packed only in apoA-I–containing HDL (Fig. 2B). Of note, in both the 35% and 65% subgroups, there was no significant change in apoCI levels and lipoprotein distribution. Although PON1 was absent in the preoperative samples of both groups, in the postoperative samples it was detectable in the heavy lipid-free fractions. In both patient subgroups, the postoperative apolipoprotein distribution was comparable to that of the control group (Fig. 3A). Semiquantitative analysis by scanning densitometry of Western blots for apoA-I, apoCIII, apoE, apoCI, and PON1 confirmed that surgical intervention resulted in a dramatic reduction in apoCIII and apoE and an increase in PON1, whereas apoCI remained unchanged, in the HDL fractions of the patients (Fig. 3B). These data further suggested an increase in the relative apoA-I/apoE HDL concentration ratio (Fig. 3C).

![Figure 1](image-url)

**Figure 1** Apolipoproteins (apo) A-I, CIII, and E distribution of lipoprotein fractions in the 35% patient subgroup. Plasma samples from each patient, were collected preoperatively (A) and postoperatively (B) and then fractionated by density gradient ultracentrifugation and analyzed for apoA-I, apoCIII, apoE, apoCI, and paraoxonase 1 (PON1) content by Western blotting, as described in the Methods. HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

<table>
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<th>Table 1 Patient characteristics pre- and postoperatively</th>
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<tr>
<td><strong>Pre group</strong></td>
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<td>35% Pre (n = 6)</td>
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<td>65% Pre (n = 11)</td>
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<tr>
<td><strong>Post group</strong></td>
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<td>35% Post (n = 6)</td>
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<td>65% Post (n = 11)</td>
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<td><strong>Pre-post</strong></td>
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<td><strong>P value</strong></td>
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<td><strong>(35%)</strong></td>
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<td><strong>(65%)</strong></td>
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<td>Age (y) 42.2 ± 3.9</td>
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<td>Males (%) 50</td>
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<td>Smokers (%) 50</td>
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<td>Body mass index (kg/m²) 55.6 ± 1.2</td>
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<td>Total cholesterol (mg/dL) 316.3 ± 24.3</td>
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<td>Triglycerides (mg/dL) 192.5 ± 37.3</td>
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<td>LDL cholesterol (mg/dL) 152.7 ± 17.4</td>
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<td>HDL cholesterol (mg/dL) 39.2 ± 4.1</td>
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<td>Non-HDL cholesterol (mg/dL) 277.1 ± 25.7</td>
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| HDL, high-density lipoprotein; LDL, low-density lipoprotein; post, postoperative; pre, preoperative.
Impact on the antioxidant potential of HDL

In an effort to determine how rapid weight loss affects HDL particle functionality, we compared the antioxidant potential of pools of HDL isolated from the 3 study groups using a modification of the dihydrorhodamine assay,22 as described in the Methods. As shown in Figure 4A, when equal amounts of HDL-C from each pool were used in the assay, the antioxidant potential of HDL 6 months after the operation was found elevated, compared with HDL isolated preoperatively or HDL isolated from the control group (all P < .005).

Electron microscopy analysis of HDL particles

To determine potential effects of surgical weight loss on the geometry and size distribution of HDL particles, we performed a qualitative negative staining EM analysis of HDL particles isolated from pools of plasma from all 3 groups following potassium bromide (KBr) density gradient UCF fractionation11 (Fig. 5A-C), as described in the Methods. This analysis showed that, in the pre group, the mean HDL particle diameter is 8.5 ± 0.1 nm, whereas the mean surface area is 46.9 ± 0.9 nm². Six months after the operation (post group), the mean HDL particle diameter increased to 8.8 ± 0.1 nm (P = .006) and the mean surface area to 54.1 ± 1.2 nm² (P < .001), reaching those of control group (mean particle diameter of 8.8 ± 0.1 nm and mean surface area of 53.0 ± 1.2 nm², both P > .05 when compared with the post group).

We further investigated the possible differences among the 3 sample groups after grouping particle diameter into 4 subgroups: smaller than 8 nm, between 8 and 10 nm, between

Figure 2 Apolipoprotein (apo) A-I, CIII, E, CI, and paraoxonase 1 (PON1) distribution of lipoprotein fractions in the 65% patient subgroup. Plasma samples from each patient were collected preoperatively (A) and postoperatively (B) and then fractionated by density gradient ultracentrifugation and analyzed for apoA-I, apoCIII, and apoE, apoCI, and PON1 content by Western blotting, as described in the Methods. HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Figure 3 Apolipoprotein (apo) A-I, CIII, and E distribution of lipoprotein fractions in control subject group (A). Plasma samples from each control subject were fractionated by density gradient ultracentrifugation and then analyzed for apoA-I, apoCIII, apoE, apoCI, and PON1 content by Western blotting, as described in the Methods. (B) The relative abundance of apolipoproteins A-I, CIII, and apoE in the 35% and 65% subgroups preoperatively and 6 months postoperatively. (C) The relative apoA-I/apoE ratio in the 35% and 65% subgroups preoperatively and 6 months postoperatively. HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.
10 and 12 nm, and larger than 12 nm but smaller than 15 nm (Fig. 5D). After a chi-squared test, it was found that the distribution in the post sample group was almost identical to that of the C sample group (P<1). However, the distribution of diameter values was significantly different between HDL particle preparations in the pre and post samples. In particular, in the pre sample group, there were more HDL particles with diameter 8 nm compared with the post sample group (P<.003, z-test), whereas in the post sample group there was a significant increase in the number of particles with diameter between 10 and 12 nm (P=.0002, z-test) (Fig. 5D).

Nondenaturing 2-dimensional electrophoresis analysis of HDL subpopulations

Our EM analysis raised the possibility that bariatric surgery promotes the formation of more mature HDL species. Therefore, in the next set of experiments we sought to determine the HDL subpopulations that were present in the 3 groups of the study. We analyzed samples from the 65% patient subgroup because the remaining 35% had barely detectable apoA-I and apoCIII in their HDL preoperatively. Pools of plasma from each group were analyzed by a classical 2-dimensional electrophoresis,25 as described in the Methods. Based on our previous work suggesting an important role of apoCIII in HDL particle functionality,24 in this analysis we determined both apoA-I–containing and apoCIII-containing HDL subpopulations. In agreement with EM results, this analysis revealed that surgery led to significant qualitative differences in the HDL subpopulations present in the pre and post sample groups (Fig. 5E-H).

In the pre group, our analysis of apoA-I HDL subpopulations indicates the presence of some pre-α2, pre-α3, pre-α4, and α3 particles, whereas most of the HDL is distributed in preβ1 immature HDL subpopulations (Fig. 5E). In the post sample group, the population of preβ1 immature HDL subpopulations appear reduced with the concomitant appearance of preβ2, preα1, α1, and α2 particles (Fig. 5F).

Similar analysis of the apoCIII-containing HDL in the pre sample group revealed the presence of some preβ1 particles, whereas most of the HDL is distributed in preα2, preα3, and preα4 particles (Fig. 5G). However, in the post group, there is an apparent reduction in the apoCIII-containing HDL subpopulations a finding consistent with the observations of Figures 2 and 3. In particular, preβ1 HDL disappears completely, whereas preα2, preα3, and preα4 particles appear reduced. Interestingly more mature preα1, α1, and α3 apoCIII-HDL particles become detectable (Fig. 5H).

Plasma LCAT and CETP activity

Given the importance of LCAT in the maturation of HDL and the conversion of pre-β HDL into α-HDL, we next hypothesized that the alterations in HDL particle subpopulation distribution that we observed 6 months after operation, may be associated with changes in plasma LCAT activity. To investigate this hypothesis, we determined LCAT activity in the pre and post sample groups, and compared it with the activity of the C group. To our surprise, results showed that LCAT activity was significantly higher in the pre samples. Six months postoperatively, the levels of plasma LCAT activity were markedly reduced compared with pre samples and similar to the activity in the C samples (Fig. 4B).

Measurement of plasma CETP activity indicated no statistically significant differences in CETP activity between preoperative and postoperative plasma samples in the 35%
subgroup (all $P > .05$) (Fig. 4C). In contrast, in the 65% subgroup, we obtained a statistically significant decrease in plasma CETP activity postoperatively. In particular, the postoperative CETP activity of this group is comparable with that of the control subjects.

**Discussion**

To investigate how rapid weight loss may influence HDL functionality and structure, in the present study we focused on patients undergoing a variant of biliopancreatic diversion. In general, malabsorptive procedures are the most effective bariatric interventions for the long-term cure of metabolic syndrome in morbidly obese patients thus far. In addition to rapid and prolonged weight loss, a main benefit of malabsorptive procedures is the normalization of plasma cholesterol profile and improvement of glucose tolerance and insulin sensitivity. Here we report that in addition to the benefits on plasma LDL-C and total cholesterol levels reported previously, rapid weight loss also results in an improvement of the antioxidant potential of HDL and a number of favorable structural changes, despite the lack of statistically significant effects on plasma HDL-C levels.

Interestingly, our initial analysis indicated that our patient cohort could be divided into 2 distinct subpopulations depending on their baseline HDL apolipoprotein composition. Specifically, we found that in 35% ($n = 7$) of our patient cohort apoA-I and apoCIII were virtually undetectable preoperatively, whereas all of their HDL cholesterol was found in apoE containing HDL particles. In contrast, in the remaining 65% ($n = 13$) of our operated patient cohort, high levels of apoA-I, apoE, and apoCIII were detected preoperatively in all HDL fractions. Postoperatively, in both patient subgroups all HDL-C was packed

![Figure 5](image-url)
exclusively in apoA-I–containing HDL particles, whereas only trace amounts of apoE and apoCIII-containing HDL particles were present. Interestingly, patient distribution between the 2 subgroups was very consistent with no gradient of phenotypes observed (supplementary data). No apparent clinical or demographic differences were recorded between the patients of the 35% subgroup and 65% subgroup. Both subgroups were clinically identical for the purpose of bariatric surgery. However, one cannot exclude that differences in the genetic profiling of the patients are responsible for the observed differences.

Epidemiological studies have suggested that the ratio of plasma apolipoproteins is a much better predictor of CHD in the general population further contributing to the theory that apolipoprotein ratios can be more informative than plasma lipid and lipoprotein levels alone.\textsuperscript{5,24,29-32} The molecular basis for this observation lays to the fact that apolipoprotein composition also defines lipoprotein functionality.\textsuperscript{35} In agreement with this theory, in the present study, in both the 35% and the 65% patient subgroups, surgical weight loss resulted in a significant increase in the apoA-I/apoE apolipoprotein ratio of HDL (Fig. 3B,C). Also, in the 65% patient subgroup, we observed a significant increase in the apoA-I/apoCIII apolipoprotein ratio of HDL postoperatively. Both of these increases were accompanied by a significant improvement in the antioxidant potential of HDL and a concomitant elevation in PON1 protein content of HDL. Unfortunately, in the case of the 35% subgroup, the complete absence of detectable apoCIII makes it impossible to define the apoA-I/apoCIII ratio preoperatively, and compare it with the postoperative ratio. Moreover, the sole presence of apoE-containing HDL in the 35% subgroup preoperatively (Fig. 1A), combined with the reduced antioxidant potential of these particles (Fig. 4A), raise the possibility that high levels of apoE-containing HDL may be a marker of dysfunctional HDL. It would be important to determine in future studies in experimental mice, how increasing the apoE content of HDL affects its antioxidant properties.

A puzzling observation associated with the malabsorptive bariatric intervention used in the present study is that patient’s HDL-C levels show a decline immediately after the operation and then start rising to preoperative levels within the first 6 months.\textsuperscript{18,33} Our data may provide a mechanistic interpretation for this observation, suggesting that the initial drop in HDL-C levels may reflect the gradual qualitative switch of HDL from apoE-containing to apoA-I–containing HDL.

Electron microscopy coupled with nondenaturing 2-dimensional electrophoresis analysis indicated that 6 months following the operation, there was a significant change in the HDL subpopulation distribution of the patients. EM indicated that, after surgery, average particle diameter and surface area of HDL increased and became comparable to the HDL isolated from the control group. These changes led us to hypothesize that the variant of biliopancreatic diversion procedure used in our study improved HDL subpopulation distribution with a shift toward more mature HDL particles. Indeed, nondenaturing 2-dimensional electrophoresis analysis confirmed this shift. We analyzed samples from the 65% patient subgroup because the remaining 35% had barely detectable apoA-I and apoCIII in their HDL preoperatively. Specifically, preoperatively a large amount of patient apoA-I–HDL was distributed in pre-β\textsubscript{1} immature HDL subpopulations, whereas 6 months postoperatively the population of pre-β\textsubscript{1} immature HDL subpopulations was significantly reduced with the concomitant appearance of mature a\textsubscript{1} and a\textsubscript{2} particles. This picture resembles the normal healthy situation where almost all HDL-C is packed in pre-α and α particles. The picture was similar for apoCIII containing HDL. Despite changes in HDL subpopulation distribution, we failed to obtain any statistically significant differences in cholesteryl efflux of pre and postoperative samples (both 35% and 65% subgroups) in an in vitro assay.

It is interesting that plasma LCAT activity in our morbidly obese patients was found elevated preoperatively, whereas 6 months later, activity was reduced to the levels of the C healthy group, showing a surprising direct correlation with the levels of pre-β HDL. One interpretation is that in our pre patient group, membrane lecithin availability becomes limited, reducing the efficacy of LCAT reaction despite elevated LCAT activity. However, another interpretation may be offered by a recent study showing that lack of LCAT expression predisposes mice to hepatic triglyceride deposition.\textsuperscript{34,35} suggesting that LCAT activity may have a protective role against processes leading to accumulation of triglycerides in the liver. Based on this observation, it is possible that the increased plasma LCAT activity in the pre group may reflect a compensatory mechanism that is engaged in an effort to combat processes leading to hepatic triglyceride accumulation and NAFLD development in the morbidly obese patients. If such a possibility exists, the exact mechanism remains to be clarified, given that LCAT activity seems to correlate mainly with hepatic triglyceride accumulation, whereas no differences are observed in hepatic free and esterified cholesterol between morbidly obese and control patients (data not shown).

Previous work by Magkos et al.\textsuperscript{36} indicated gender-specific differences in the activity of lipid transfer proteins in nondiabetic lean and obese men and women. In our experiments, we did not observe such gender-specific differences in the structural alterations of HDL or in LCAT activity, either preoperatively or 6 months postoperatively. One possible reason for this difference is that our patients were at the rapid weight loss stage 6 months after the operation. It is possible that after few years of sustained weight loss, differences in HDL structure and lipid transfer protein activity may appear between genders in our patient cohort. No apparent clinical or demographic differences were recorded between the patients of the 35% subgroup and 65% subgroup. Both subgroups were clinically identical for the purpose of bariatric surgery. However, one cannot exclude that differences in the genetic profiling of the patients are responsible for the observed differences.
Interestingly, measurement of plasma CETP activity indicated no statistically significant differences in CETP activity between pre and postoperative plasma samples in the 35% subgroup (all $P > 0.05$) (Fig. 4C). In contrast, in the 65% subgroup, we obtained a statistically significant decrease in plasma CETP activity postoperatively. In particular, the postoperative CETP activity of this group is comparable with that of the control subject. Given the role of both apoA-I and apoE as activators of CETP in plasma, it is possible that these observations are explained by the different plasma apoA-I and apoE content in the pre- and postoperative states of the 35% and 65% patient subgroups. In particular, in the 35% subgroup, we have an almost quantitative replacement of apoE preoperatively by apoA-I postoperatively. However, in the 65% subgroup, we obtained a total elimination of apoE in the postoperative plasma accompanied by a concomitant reduction in apoA-I levels. Therefore, in the 65% subgroup, there is an apparent reduction in the concentration of CETP activators that possibly translates to reduced CETP activity.

Because apoCI is a potent modulator of plasma LCAT and CETP activities, one could speculate that the observed differences in the activities of these 2 enzymes may be attributed to changes in apoCI levels. Analysis of apoCI by Western blotting in the lipoprotein fractions pre- and postoperatively for both the 35% and 65% subgroups indicated no significant alterations in apoCI levels and lipoprotein distribution, suggesting that the differences in the activities of both LCAT and CETP pre- and postoperatively are independent of apoCI and may be simply due to the changes of apoA-I and apoE content in plasma.

In addition to the normalization of plasma cholesterol levels reported previously, our data suggest a significant alteration in HDL structure that is accompanied by an increase in its antioxidant potential. In particular, our data raise the possibility that the apoA-I/apoE and apoA-I/apoCIII concentration ratios in HDL may serve as surrogate markers of HDL functionality, although no recommendation can be made before this statement is confirmed in a prospective clinical trial. Overall, it appears that rapid weight loss exerts a beneficial effect on HDL quality. It would be useful and clinically relevant to determine, a few years after surgical weight loss, when body weight has been stabilized, how sustainable these changes in HDL quality are and how they may translate to long-term atheroprotection.

It is generally accepted that lifestyle interventions and physical activity are important parameters in improving HDL functionality and sedentary lifestyle may have the opposite effects. Our study did not assess separately the effects of physical activity on HDL structure and function. However, as BMI is progressively reduced after surgery, physical activity is expected to increase. It would be very interesting to compare how the effects of rapid weight loss on HDL structure and function that we observed in this study relate to those after restrictive bariatric procedures or exercise- and diet-induced weight loss.

Acknowledgments

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jacli.2014.05.001.

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