Relationship Between Cholesteryl Ester Transfer Protein and Atherogenic Lipoprotein Profile in Morbidly Obese Women


Objective—Obesity is associated with increased morbidity and mortality from atherosclerotic disease. Lipid abnormalities contribute to the increased relative risk in obese subjects. Cholesteryl ester transfer protein (CETP) mass is increased in these patients and might mediate the atherogenic lipoprotein pattern observed in obesity.

Methods and Results—Twenty-one morbidly obese, middle-aged, female subjects participated in this prospective study. Subjects were examined before and 1 year after surgical treatment. Fat mass was determined by body impedance analysis; CETP mass, by ELISA; CETP activity, by exogenous substrate assay; and LDL particle diameter, by gradient gel electrophoresis. Mean weight loss after 1 year was 28.7 kg; mean fat mass loss was 22.6 kg. Mean CETP mass decreased from 1.81 to 1.32 μg/mL (P=0.008); mean CETP activity decreased from 244 to 184 nmol · mL⁻¹ · h⁻¹ (P=0.004); and in parallel, the mean diameter of LDL particles increased (256.8 to 258.4 Å, P=0.04).

Conclusions—We conclude that weight loss is associated with a pronounced decrease in CETP mass and activity and a consistent increase in LDL particle diameter. After 1 year of this prospective study in morbidly obese subjects undergoing weight loss by surgical treatment, it has been determined that some features of the atherogenic lipoprotein profile can be reversed. (Arterioscler Thromb Vasc Biol. 2002;22:●●●●.●●●●.)

Key Words: obesity ■ gastric banding ■ lipids ■ cholesteryl ester transfer protein

Obesity is linked to a variety of metabolic and hormonal dysfunctions, such as the development of insulin resistance and dyslipidemia, leading to increased morbidity and mortality in these subjects. Adipose tissue is not solely an energy storage tissue but is also involved in metabolic processes expressing and secreting bioactive compounds, such as cholesteryl ester transfer protein (CETP) and leptin.

Obesity is associated with dyslipidemia, elevated triglyceride (TG) levels, low HDL cholesterol (HDL-C) levels, and small dense LDL particles. These alterations in plasma lipoprotein lipid metabolism have been shown to increase the risk of cardiovascular disease in men and women. The state of dyslipidemia is also influenced by body fat distribution. Abdominal obesity is linked to a higher risk profile for cardiovascular disease.

Adipose tissue is a prominent source of CETP. CETP is an important determinant of lipoprotein composition because of its capacity to mediate the transfer of cholesteryl esters (CEs) from CE-rich lipoproteins to TG-rich lipoproteins in exchange for TGs. In obese subjects, CETP activity and mass were increased, whereas lipoprotein lipase (LPL) activity after heparin administration was significantly decreased.

LDL particles vary in size, density, and composition. LDL particles can be classified into 2 LDL subclass patterns: pattern A describes the preponderance of large LDL particles, whereas small dense LDL particles are defined as pattern B. In several epidemiological studies, the presence of small dense LDL particles has been linked to atherosclerotic diseases. Several cellular and molecular mechanisms have been implicated in the higher atherogenicity of pattern B LDL particles. Compared with pattern A LDL particles, small dense pattern B particles have been shown to be more accessible to oxidative modification and to bind with higher affinity to vascular proteoglycans. Small dense LDL particles also have a reduced affinity for the LDL receptor because of the conformational changes in apoB found on small LDL particles; thus, they have a prolonged residence time in plasma (see review). In the present study, we evaluated whether the atherogenic lipoprotein profile associated with obesity is reversed by massive weight reduction and whether such a reversion occurs in parallel with a CETP mass diminution.

Methods

Subjects Twenty-one morbidly obese women, defined by a body mass index (BMI) of >40 kg/m², participated in this prospective study. Exclusion criteria were secondary causes of adiposity, diabetes mellitus,
pregnancy, intake of lipid-lowering drugs, or other medically significant illness. Examinations of the study subjects were undertaken within 2 months before Swedish adjustable gastric banding (SAGB) and 1 year after SAGB. Informed consent was obtained before entering into the study, and all procedures were performed in accordance with institutional guidelines at the Internal Department of the Medical Faculty of the University of Innsbruck.

Surgical Procedure
The surgical procedures were performed as described by Forsell et al. at the Department of Surgery, University of Innsbruck. The SAGB (SAGB Obtech Medical AG) was used in all of the study patients.10

Analysis of Body Composition
BMI was calculated as body weight in kilograms divided by height in meters squared. Body composition was determined by impedance analysis with the use of a multifrequency BIA 2000-M Impedance Analyser (Data Input). Silver/silver chloride electrodes were placed on the surface of the right ankle and on the right wrist of the patient, and resistance and reactance were measured by using 1, 5, 50, and 100 kHz, respectively. Measurements were taken in the morning after each participant had fasted at least 8 hours. Fat-free mass and fat mass were determined by using Nutri 4 software (Data Input).

Lipoprotein Analysis
Blood was collected after an overnight fast. Plasma was separated from erythrocytes by centrifugation at 3000 rpm for 10 minutes at 4°C immediately after collection. Plasma samples were stored frozen at −80°C until they were assayed. Plasma TG, cholesterol, and apoA-I and apoB concentrations were quantified by using a commercially available enzymatic kit (Roche Diagnostic Systems) on a Cobas Mira analyser. HDL-C and HDL3-C concentrations were determined by using precipitation procedures with polyethylene glycol (Immuno). HDL3-C concentrations were calculated by subtracting HDL-C from HDL3-C. LDL cholesterol was calculated according to the formula of Friedewald et al.11

CETP Mass
CETP concentrations were determined by capture ELISA. Wells were coated with recombinant single-chain antibody fragments 1CLR.12 CETP was detected by using a polyclonal anti-CETP antibody conjugated directly to alkaline phosphatase.13

CETP Activity
CETP activity was determined as the transfer of radiolabeled CE from exogenous LDL to exogenous HDL in lipoprotein-depleted plasma.14 CETP activity is expressed as nanomoles per milliliter per hour CE transfer from LDL to HDL2.

LDL Particle Size
LDL size was analyzed by 0.75% to 16% gradient polyacrylamide gel electrophoresis of whole plasma (Labomed). Gels were fixed and stained with Sudanblack (Labomed). Well-characterized control samples were run on each gel in parallel and used to construct a quadratic calibration curve to estimate the diameter of the major peak of LDL particles.15,16

Statistical Analysis
Descriptive data are expressed as mean±SD. The data from the pre- and post–gastric banding subjects were compared by using a paired-sample t test. Statistical significance was inferred at a 2-tailed value of P<0.05. Statistical analyses were calculated by using SPSS release 8.0 for Windows.

Results
Baseline Characteristics
Apolipoproteins
ApoA-I and apoB levels decreased from baseline concentrations of 141±15 and 114±26 mg/dL, respectively, to 138±19 and 102±18 mg/dL, respectively, 1 year after SAGB. The change of apoA-I was not significant, whereas a statistically significant decrease of apoB was demonstrated (P<0.004, Table 2).

Plasma CETP Concentrations and CETP Activity
The CETP mass was 1.81±0.96 μg/mL at baseline, corresponding to a fat mass of 55.1 kg per individual studied. After a weight loss of 22.6 kg fat mass after gastric banding, CETP mass decreased to 1.32±0.71 μg/mL (P=0.008, Table 2). CETP activity decreased with weight loss from 244±64 to 184±61 nmol · mL−1 · h−1 (P=0.004, Table 2).

LDL Pattern Analysis
LDL particle size, as determined by a polyacrylamide gel electrophoresis method, increased from baseline levels of
256.8±7.2 Å to 258.4±6.9 Å at 1 year after the surgical procedure (P=0.035, Table 2). The Figure shows LDL particle size in pre- and post-gastric banding subjects.

### Discussion

Obesity is associated with a higher risk of morbidity and mortality from atherosclerotic disease.1 Bariatric surgery is an efficient method for reducing body weight. Major weight loss leads to beneficial metabolic effects, such as lowering TGs, improving the insulin-to-glucose ratio, and increasing HDL-C. A 4-year follow-up of obese patients treated with weight-reducing gastroplasty compared with an obese control group showed improvements in HDL-C, TG, and insulin levels.17

CETP has a key role in reverse cholesterol transport, that of mediating the transfer of CE from CE-rich lipoproteins to TG-rich lipoproteins in exchange for TGs. This transfer reaction alters the chemical, physical, and biological properties of the respective lipoprotein particles. Evidence that CETP may be antiatherogenic has been reported in human18 and animal19,20 studies. A deficiency of CETP protein in humans leads to the formation of large apoE-enriched HDL, and to an increase of HDL-C, which is associated with reduced incidence of coronary heart disease.21 On the other hand, expression of CETP in atherosclerosis-prone mice and rabbits has been reported to increase the incidence of atherosclerosis in these animals.22-24

White adipose tissue is a secretory organ producing a variety of substances playing a role in immunologic responses, vascular disease, and appetite regulation, such as leptin, adipin, acylation stimulation protein, tumor necrosis factor-α, and migration inhibitory factor.25 Adipose tissue is a major source of CETP expression.4 CETP mRNA levels have been positively correlated with plasma CETP concentrations, as determined recently in a number of patients undergoing coronary artery bypass grafting,26 suggesting that experiments using quantitative mRNA techniques and CETP protein concentrations can be compared.

During differentiation of human fibroblastic preadipocytes to adipocytes in primary cell culture, compared with LPL mRNA and adipsin mRNA, CETP mRNA was detected at an even earlier stage of differentiation; these mRNAs are all markers of late differentiation.27 CETP mRNA is most highly expressed in immature fat cells of the human adipose tissue.28

White adipose tissue formation begins before birth, and expansion takes place rapidly after birth as a result of increased fat cell size as well as fat cell number. Also, fat cell number increases in adult life in severe human obesity. The studies of Gauthier et al27 and Radeau et al28 suggest that CETP levels might be increased in neonates and in children undergoing rapid weight gain. On the other hand, early differentiation markers can be detected even in adipose tissue derived from very old mice.29 Moreover, fat cell precursors isolated from adult white adipose tissue of various species, including humans, can be differentiated in vitro into mature adipocytes.30

LDL particles, which are the major cholesterol-carrying lipoproteins in plasma, vary in size, density, and composition. Small dense LDL particles are associated with the development of non-insulin-dependent diabetes mellitus.31 Although small dense LDL particles are genetically influenced, body fat composition seems to affect the LDL phenotype marked-

### Table 2. Changes in Lipid Parameters, Apolipoproteins, LDL Particle Size, and Plasma CETP Mass at Baseline and After Weight Loss Induced by SAGB

<table>
<thead>
<tr>
<th></th>
<th>Pre SAGB</th>
<th>Post SAGB</th>
<th>Test Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>194±35</td>
<td>198±31</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>135±44</td>
<td>99±34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>114±34</td>
<td>122±28</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>53±8</td>
<td>57±15</td>
<td>NS</td>
</tr>
<tr>
<td>HDL2-C, mg/dL</td>
<td>15±6</td>
<td>19±8</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL3-C, mg/dL</td>
<td>43±8</td>
<td>43±11</td>
<td>NS</td>
</tr>
<tr>
<td>Apo-AI, mg/dL</td>
<td>141±15</td>
<td>137±19</td>
<td>NS</td>
</tr>
<tr>
<td>Apo-B, mg/dL</td>
<td>114±26</td>
<td>102±18</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL particle size, Å</td>
<td>256.8±7.2</td>
<td>258.4±6.9</td>
<td>0.035</td>
</tr>
<tr>
<td>CETP, mg/mL</td>
<td>1.81±0.96</td>
<td>1.32±0.71</td>
<td>0.008</td>
</tr>
<tr>
<td>CETP activity, nmol - mL⁻¹ - h⁻¹</td>
<td>244±64</td>
<td>184±61</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD. Statistical significance was estimated by paired-samples t test. NS indicates not significant.

LDL particle size in pre- and post-gastric banding subjects. Lanes 1 to 4 present the LDL particle size of 4 study subjects before (a) and after (b) gastric banding. Lane 5 shows standards for LDL pattern A (a) and LDL pattern B (b).
ly.\textsuperscript{2} In the Framingham study population, the percentage of LDL pattern B increased with rising BMI.\textsuperscript{2} Weight reduction induced by caloric restriction has been shown to lead to an increase in the LDL particle size in older men with pattern B at baseline.\textsuperscript{32} Furthermore, LDL particle size has been shown to be inversely correlated with plasma TG concentration.\textsuperscript{33} Hypertriglyceridemia by itself is associated with an elevated occurrence of cardiovascular disease.\textsuperscript{34,35} Young women with dense LDL particles of a smaller size are at a higher risk of early-onset myocardial infarction than are control subjects with larger and lighter LDL particles.\textsuperscript{36}

The beneficial effects of weight reduction on the atherogenic lipoprotein profile in obese subjects are well established.\textsuperscript{37} The present study confirms and extends the results of previous studies. Pronounced weight loss induced by surgical intervention induced a significant decrease in plasma CETP concentrations in middle-aged women. One year after gastric banding, fat mass decreased by 41\%, and in parallel, CETP mass decreased by 27\%. In addition, TGs decreased by 27\%. LDL particle diameters increased significantly after the study period. Furthermore, HDL\textsubscript{2}-C increased by 4 mg/dL, and LDL particle diameters increased significantly after the study period. Furthermore, HDL\textsubscript{2}-C increased by 4 mg/dL, and apoB decreased by 12 mg/dL. Also, the ratio of LDL cholesterol to apoB increased, suggesting an increase of LDL particle cholesterol content and an increase in LDL particle diameter. Thus, the results of our prospective study indicate that the stigmata of the atherogenic lipoprotein profile, ie, small dense LDL particles and small HDL\textsubscript{3}, and high levels of CETP, are reversible.

Several genes are considered as candidates influencing the LDL size distribution: the LDL receptor,\textsuperscript{38} manganese superoxide dismutase,\textsuperscript{38} the apoA-I-C3-A4 gene locus,\textsuperscript{39} CETP,\textsuperscript{40} lecithin-cholesterol acyltransferase,\textsuperscript{39} and apoB.\textsuperscript{41}

In addition, Purnell et al.\textsuperscript{32} demonstrated a decrease in the activity of postheparin hepatic lipase in 21 obese older men after weight loss, suggesting that the reduced hepatic lipase concentrations might mediate the improvement in the lipoprotein profile. CETP activity was determined, although no changes in CETP activity were noted after weight loss in the study by Purnell et al. Several reasons could contribute to the potentially conflicting results between these studies: (1) In the present study, middle-aged women were investigated, whereas the study population of Purnell et al consisted of older men. (2) Fat distribution differs substantially between women and men. In vitro studies have demonstrated a more pronounced expression of CETP mRNA in subcutaneous adipose tissue.\textsuperscript{42} (3) In the present study, the women underwent a weight loss of ≈30 kg, whereas in the study of Purnell et al, men lost an average of 10 kg.

Evidence from clinical studies support a role of CETP in LDL size regulation by exchanging CE from HDL- to apoB-containing lipoproteins.\textsuperscript{40} Furthermore, in vitro studies have demonstrated that lipolysis of lecithin-cholesterol acyltransferase and CETP-reacted plasma by LPL results in an alteration in LDL density.\textsuperscript{43} Increasing TG contents led to an enhancement of this effect, resulting in the production of small dense LDL.

However, the strongest evidence for CETP as a major determinant of LDL size comes from recent studies by Austin et al.\textsuperscript{44} and Talmud et al.\textsuperscript{45} These studies conclusively con-firmed linkage of LDL size to the CETP gene, whereas they did not provide significant evidence of a genetic linkage with some of the other candidate genes.

Of course, the individual TG metabolic capacity is also a strong determinant of the LDL size and of HDL\textsubscript{C}, in particular, HDL\textsubscript{2}-C levels.\textsuperscript{46} Although we determined only the fasting TG levels and not postprandial lipemia in the study subjects, the influence of the TG metabolic capacity is clear from previous studies.

In conclusion, we found a marked reduction of CETP mass in subjects undergoing pronounced weight loss after gastric banding, and the present study supports a role of CETP in the reversibility of the atherogenic lipoprotein profile seen in obese subjects. During marked reduction in the fat mass, the serum levels of its product, CETP, are reduced in parallel. This prospective study suggests that the diminution of the CETP concentration may play a favorable role in the improvement of the lipoprotein profile after pronounced weight loss.

Acknowledgment

This study was supported by grant 9442 of the Österreichische Nationalbank.

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Arterioscler Thromb Vasc Biol. published online August 1, 2002;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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