Hypertriglyceridemia is associated with preβ-HDL concentrations in subjects with familial low HDL

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Abstract Preβ-HDL particles act as the primary acceptors of cellular cholesterol in reverse cholesterol transport (RCT). An impairment of RCT may be the reason for the increased risk of coronary heart disease (CHD) in subjects with familial low HDL. We studied the levels of serum preβ-HDL and the major regulating factors of HDL metabolism in 67 subjects with familial low HDL and in 64 normolipidemic subjects. We report that the subjects with familial low HDL had markedly reduced preβ-HDL concentrations compared with the normolipidemic subjects (17.4 ± 7.2 vs. 23.4 ± 7.8 mg apolipoprotein A-I/dl; $P < 0.001$). A positive correlation was observed between preβ-HDL concentration and serum triglyceride (TG) level ($r = 0.334$, $P = 0.006$). In addition, serum TG level was found to be the strongest predictor of preβ-HDL concentration in subjects with familial low HDL. The activities of cholesteryl ester transfer protein and hepatic lipase were markedly increased in subjects with familial low HDL without a significant correlation to preβ-HDL concentration. Our results support the hypothesis that impaired RCT is one mechanism behind the increased risk for CHD in subjects with familial low HDL.—Söderlund, S., A. Soro-Paavonen, C. Ehnholm, M. Jauhiainen, and M-R. Taskinen. **Hypertriglyceridemia is associated with preβ-HDL concentrations in subjects with familial low HDL. *J. Lipid Res.* 2005.** 46: **1643–1651.**

Supplementary key words reverse cholesterol transport • coronary heart disease • hepatic lipase • phospholipid transfer protein • cholesteryl ester transfer protein • insulin resistance • metabolic syndrome • apolipoprotein A-I • high density lipoprotein

Plasma levels of HDL cholesterol correlate inversely with the risk of coronary heart disease (CHD). The precise mechanism of this effect is not fully established, although its role in the reverse cholesterol transport (RCT) process is well recognized (1, 2). This pathway involves the uptake of cholesterol from peripheral cells and the subsequent transport and delivery of the cholesterol to the liver for excretion in a form of bile acids or free cholesterol (FC). The initial extracellular acceptors of cellular cholesterol are small lipid-poor apolipoprotein A-I (apoA-I) particles (3). These particles, termed preβ-HDL because of their electrophoretic mobility, represent either discoidal particles, consisting of two or three molecules of apoA-I complexed with phospholipids and unesterified cholesterol, or lipid-free apoA-I (4, 5). The efflux of phospholipids (sphingomyelin and phosphatidylcholine) and unesterified cholesterol from cell membranes to preβ-1 HDL via ABCA1 is the first step in the RCT pathway (6–8). In the next step of RCT, LCAT esterifies FC taken up by preβ-HDL and preβ-HDL is transformed to spherical α-HDL containing a cholesteryl ester core (4).

The reported concentration of preβ-migrating apoA-I varies widely, partly because of biological variation but also because of differences in the techniques used to quantify these particles. Most studies of preβ-migrating apoA-I have not differentiated between discoidal preβ-HDL and monomolecular lipid-free/poor apoA-I (9). There is evidence from in vitro studies that lipid-free/poor apoA-I is generated during the remodeling of HDL and that this form of apoA-I may play a fundamental role in the efflux of cholesterol from macrophage foam cells. So far, data on the existence of lipid-free/poor apoA-I in plasma in vivo is somewhat controversial (9, 9a).

Phospholipid transfer protein (PLTP) is an important regulator of HDL metabolism, which is attributable to its phospholipid transfer activity (10, 11) inducing HDL conversion in a process that remodels HDL size and composition (12–14). PLTP is able in vitro to generate preβ-HDL by converting HDL_3 particles into larger fusion particles with concomitant release of lipid-poor apoA-I, which dis-

Manuscript received 7 December 2004 and in revised form 17 February 2005 and in re-revised form 13 April 2005.

Published, JLR Papers in Press, May 16, 2005.
DOI 10.1194/jlr.M400480-JLR200

Abbreviations: apoA-I, apolipoprotein A-I; BMI, body mass index; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; FC, free cholesterol; HDL-C, high density lipoprotein-cholesterol; HOMA IR, homeostasis model assessment for insulin resistance; LpA-I, lipoprotein A-I; LpA-I/A-II, lipoprotein A-I/A-II; PLTP, phospholipid transfer protein; RCT, reverse cholesterol transport; TC, total cholesterol; TG, triglyceride; W/H ratio, waist/hip ratio.

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plays preβ mobility in agarose electrophoresis (14–16). PLTP is also capable of promoting the formation of preβ-HDL particles from HDL_2 (17). Moreover, in vivo data from mice overexpressing human PLTP demonstrate that their plasma has an increased capacity to produce preβ-HDL (15, 18, 19). It has been suggested that PLTP can act as an antiatherogenic factor by preventing cellular cholesterol overload by the generation of preβ-HDL (15). PLTP-generated preβ-HDL particles have been demonstrated to be responsible for the increased efflux of cholesterol from cultured fibroblasts (20) and macrophages; thus, PLTP has an antiatherogenic function by contributing to the removal of cholesterol from lesion macrophages (21). Direct evidence of the interaction of PLTP with macrophage ABCA1 and enhanced cholesterol efflux to HDL in the presence of PLTP was recently reported by Oram et al. (22).

Preβ-HDL formation can also be promoted by HL. It induces the formation of preβ-like HDL particles upon incubation with both native and triglyceride (TG)-enriched HDL_2 (17, 23, 24). Another pathway of preβ-HDL particle formation is cholesteryl ester transfer protein (CETP)-induced HDL conversion. The incubation of HDL with CETP in the presence of VLDL gives rise to preβ-HDL generation (25). CETP also produces TG-enriched HDL, which is a preferred substrate for HL. Apparently, CETP and HL act in concert to promote HDL transformation and preβ-like HDL formation (17).

Given that preβ-HDL has a crucial role in RCT by acting as the primary acceptor of cellular cholesterol (3), it may contribute to the protective effects of HDL. To date, data on the amount of preβ-HDL in subjects with familial dyslipidemic syndromes or in normolipidemic healthy subjects with no lipid abnormalities are sparse. Therefore, we studied the levels of serum preβ-HDL and the major regulating factors of HDL metabolism in subjects with familial low HDL. In addition to serum preβ-HDL quantitation, we measured PLTP concentration, the activities of CETP and PLTP, and postheparin plasma activities of HL and LPL in 131 subjects: 67 family members from 35 carefully characterized Finnish families with familial low HDL and premature CHD, and 64 normolipidemic control subjects.

MATERIALS AND METHODS

Study subjects

The carefully characterized pedigrees with low HDL-cholesterol (HDL-C) and premature CHD were recruited in Helsinki and Turku University Central Hospitals in Finland as described previously (26, 27). Briefly, the low-HDL probands were required to be 30–60 years old and to have CHD verified by either coronary angiography (at least 50% stenosis in one or more coronary arteries) or myocardial infarction, HDL-C level below the 10th age/sex-specific Finnish population percentile (<0.9 mmol/l in men and <1.1 mmol/l in women), TG < 2.3 mmol/l, and total cholesterol (TC) < 6.3 mmol/l in men and <6.0 mmol/l in women. Of the 35 low-HDL families enrolled in this study, 67 family members who were defined as affected based on HDL-C below the 10th percentile level were included. Each of these 35 low-HDL families contributed at least one affected subject to the study. In the affected group, 31 subjects had CHD, of whom 30 were probands. In five families, we could not access the proband at the time when the blood samples for this approach were taken. Affected subjects who did not live a reasonable distance from Helsinki or who used medications potentially affecting HDL metabolism at the time the samples were taken were excluded. The control group consisted of 64 subjects: 42 normolipidemic healthy volunteers and 22 healthy spouses from low-HDL families. Any lipid-lowering medication was discontinued for 4 weeks before the blood sampling. Each study subject gave written informed consent before participating in the study. All samples were collected in accordance with the Helsinki declaration, and the ethics committees of the participating centers approved the study design.

Biochemical analyses and demographic variables

Venous blood samples were obtained after an overnight fast. Serum and EDTA plasma were separated by centrifugation and stored at −80°C until analysis. Serum TC and TG were determined with an automated Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) by enzymatic methods (Hoffman-La Roche kits 0722138 and 0715166, respectively). Serum HDL-C was quantified by phosphotungstic acid/magnesium chloride precipitation procedures (Hoffman-La Roche kit 0720674). Serum LDL was calculated from the Friedewald formula [LDL = TC − (HDL-C) − TG/2.2] (28). The concentrations of apoA-I, apoA-II, and apoB were measured by immunoturbidometric methods with commercial kits (Boehringer-Mannheim, Mannheim, Germany), and lipoprotein A-I (LpA-I) particles were quantitated by differential electroimmunoassay (Sebia, Issy-les-Moulienaux, France) (29). The concentration of lipoprotein A-I/A-II (LpA-I/A-II) particles was calculated by subtracting the concentration of LpA-I from the total concentration of apoA-I in serum. HDL_2 and HDL_3 were separated by ultracentrifugation, and HDL composition was analyzed as described previously (30). Plasma glucose concentrations were analyzed by the glucose dehydrogenase method (Precision-G Blood Glucose Testing System; Medisense, Abbott). Serum free insulin concentrations were measured by radioimmunoassay (Phadeseph INSULIN RIA; Pharmacia and Upjohn, Uppsala, Sweden). The homeostasis model assessment for insulin resistance (HOMA IR) was calculated from the fasting plasma glucose and serum insulin concentrations as follows: fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5 (31, 32). The quantification of preβ-HDL was performed by crossed immunoelectrophoresis (15). The preβ-HDL area is expressed as a percentage of the sum of α-HDL and preβ-HDL areas. Preβ-HDL concentration is given in absolute amount [apoA-I present in preβ-HDL particles (mg/dl serum)]. In preβ-HDL assays, the average coefficient of variation was 14%. After separation of serum and plasma by centrifugation, they were immediately frozen and stored at −80°C until preβ-HDL analysis, and no LCAT inhibitors, either iodoacetamide or DTNB, were added. Antibodies used in the assay were raised in New Zealand White rabbits against a highly purified human apoA-I obtained from Dr. Peter Lerch (Swiss Red Cross, Bern, Switzerland). The method of apoA-I purification has been described (33). Rabbit antibodies reacted only with human apoA-I when analyzed with SDS-PAGE Western blot analysis. Postheparin plasma LPL and HL activities were measured as described (34). CETP activity was measured by the method of Groener, Pelton, and Kostner (35). PLTP activity was measured using the radiometric assay described by Damen, Regts, and Scherphof (36) with minor modifications (14, 36, 37). PLTP protein concentration was measured using a recently reported PLTP ELISA method (38).

Hypertension was defined as systolic blood pressure ⩾ 140 mm Hg, diastolic blood pressure ⩾ 90 mm Hg, or self-reported use of antihypertensive medications. Each participant filled out a

standard questionnaire on medication, drinking, and smoking habits. The smoking status of the subjects was categorized in two groups, smokers and nonsmokers.

Statistical methods

Statistical comparisons of clinical and biochemical parameters were performed with SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL). Results are expressed as means ± SD for continuous variables and as frequencies or percentages for categorical variables. Men and women were coded with the nominal variables 1 and 2, respectively. The normality of each variable distribution was tested by the Kolmogorov-Smirnov test, and variables with skewed distribution were $\log_{10}$ transformed before analyses, but the values in text, tables, and figures are presented as nontransformed. The following measurements demonstrated a nonnormal distribution: preβ-HDL percentage and concentration, apoA-I, LpA-I, LpA-I/A-II, TG, VLDL-TG, HDL_2-C, LPL, and HL/LPL ratio. The distributions of preβ-HDL percentage and concentration were similar to those described earlier by O'Connor et al. (39). Continuous variables were compared between the groups by general linear model, univariate ANOVA. $P < 0.05$ was considered significant (two-tailed). The frequency distribution of the categorical variables was compared between the groups with the Chi-square test. The relationships of biochemical and clinical characteristics were examined by Pearson's correlation and Spearman correlation analysis, as appropriate. Multivariate stepwise linear regression analysis was performed to determine the relative contribution of different parameters to the preβ-HDL concentration in the affected family members and control subjects. Independent variables were removed from the model until the best-fitting model with the maximum adjusted multiple R^2 was achieved. ApoA-I and LpA-I/A-II were not included in the model because the amount of apoA-I is used in the calculation of the preβ-HDL concentration. Insulin and glucose were not included in the same model with HOMA IR because these were highly intercorrelated. For the same reason, HDL-C, HDL_2-C, HDL_3-C, apoA-II, and LpA-I were not included in the same model. In addition, LDL and TC were not included in the same model because LDL was calculated from the Friedewald formula (28).

RESULTS

The clinical and biochemical characteristics of the study subjects are presented in **Tables 1**, **2**. Age distribution was similar between the affected low-HDL family members and the control subjects. Thirty-one affected low-HDL family members had CHD and 36 low-HDL family members were free of CHD. The percentage of hypertensive subjects was higher among the affected low-HDL family members. The affected family members had significantly higher body mass index [BMI; weight/height squared (kg/m^2)], waist/hip ratio (W/H ratio), fasting insulin and glucose levels, and HOMA IR than the control subjects. They also had higher levels of TG, VLDL-TG, and apoB.

The affected family members had lower preβ-HDL concentrations [apoA-I present in preβ-HDL particles (mg/dl serum)] than the control subjects. In addition, the relative amount of preβ-HDL (percentage of apoA-I in preβ-HDL particles) was significantly lower among the affected family members. By definition, HDL-C was significantly lower in the affected low-HDL family members than in the control subjects [mean 0.85 ± 0.16 vs. 1.48 ± 0.34 (mmol/l); $P < 0.001$]. Likewise, the levels of HDL_2-C, HDL_3-C, apoA-I, apoA-II, LpA-I, and LpA-I/A-II were all lower in the low-HDL family members compared with those in the control subjects.

The affected men had significantly less preβ-HDL than the affected women (16.6 ± 7.2 vs. 21.1 ± 6.4 mg apoA-I/dl; $P = 0.0430$). The affected men also had significantly less preβ-HDL than the control men (16.6 ± 7.2 vs. 24.6 ± 6.0 mg apoA-I/dl; $P < 0.001$). Next, the low-HDL family members were allocated into two subgroups based on their CHD status. The CHD patients tended to have less preβ-HDL than the family members free of CHD. The difference was seen in both relative (13.9 ± 4.6 vs. 15.5 ±

TABLE 1. Clinical and biochemical characteristics of the study subjects

Variable	Affected Low-HDL Family Members (n = 67; 55 men/12 women)	Affected Low-HDL Family Members with CHD (n = 31; 30 men/1 woman)	Control subjects (n = 64; 42 men/22 women)
Age (years)[a]	49.6 ± 12.2	57.7 ± 5.9	50.1 ± 11.7
CHD, n (%)[a]	31 (46.3%)[c]	31 (100%)	0
Hypertensive, n (%)[a]	19 (28.4%)[d]	13 (41.9%)	4 (6.3%)
Smokers, n (%)[a]	16 (23.9%)	5 (16.1%)	11 (17.2%)
BMI (kg/m^2)[b]	27.4 ± 3.7[c]	27.0 ± 2.0	25.0 ± 3.3
Waist/hip ratio	0.93 ± 0.08[d]	0.95 ± 0.05	0.88 ± 0.07
Systolic blood pressure (mm Hg)	130 ± 17	136 ± 17	126 ± 17
Diastolic blood pressure (mm Hg)	82 ± 10	84 ± 8	80 ± 9
Glucose (mmol/l)	5.2 ± 0.9[d]	5.2 ± 0.8	4.8 ± 0.6
Insulin (mU/l)	10.9 ± 5.8[c]	11.3 ± 4.7	5.6 ± 3.2
HOMA IR	2.5 ± 1.6[c]	2.5 ± 1.2	1.2 ± 0.8
TC (mmol/l)	5.1 ± 0.9	5.2 ± 0.7	5.0 ± 0.8
TG (mmol/l)	1.7 ± 0.9[c]	1.6 ± 0.6	1.0 ± 0.3
VLDL-TG (mmol/l)	1.06 ± 0.69[c]	1.05 ± 0.54	0.54 ± 0.29
LDL (mmol/l)	3.5 ± 0.8	3.7 ± 0.6	3.1 ± 0.8
ApoB (mg/dl)	105.4 ± 25.6[d]	110.2 ± 19.4	90.0 ± 19.9

Data are presented as means ± SD. apoB, apolipoprotein B; BMI, body mass index; CHD, coronary heart disease; HOMA IR, homeostasis model assessment for insulin resistance; TC, total cholesterol; TG, triglyceride.

[a] *P* values for age, CHD, hypertension, and smoking are not adjusted.

[b] *P* values calculated for BMI are adjusted for age and sex.

[c] Sex-, age-, and BMI-adjusted *P* value between affected low-HDL family members and control subjects was <0.001.

[d] Sex-, age-, and BMI-adjusted *P* value between affected low-HDL family members and control subjects was <0.050.

TABLE 2. Biochemical characteristics of the study subjects

Variable	Affected Low-HDL Family Members (n = 67; 55 men/12 women)	Affected Low-HDL Family Members with CHD (n = 31; 30 men/1women)	Control Subjects (n = 64; 42 men/22 women)
HDL-C (mmol/l)	0.85 ± 0.16[a]	0.80 ± 0.13	1.48 ± 0.34
HDL_2-C (mmol/l)	0.34 ± 0.14[a]	0.33 ± 0.14	0.70 ± 0.31
HDL_3-C (mmol/l)	0.64 ± 0.11[a]	0.61 ± 0.10	0.84 ± 0.16
ApoA-I (mg/dl)	119 ± 42[a]	120 ± 60	143 ± 23
ApoA-II (mg/dl)	31.9 ± 4.3[a]	30.7 ± 4.0	36.5 ± 7.4
LpA-I (mg/dl)	36.0 ± 11.2[a]	34.8 ± 7.8	52.5 ± 15.7
LpA-I/A-II (mg/dl)	82.5 ± 42.7[b]	84.9 ± 61.7	90.5 ± 16.7
Relative preβ-HDL (% of plasma apoA-I)	14.8 ± 5.2[b]	13.9 ± 4.6	16.4 ± 4.7
Absolute preβ-HDL (mg apoA-I/dl)	17.4 ± 7.2[a]	16.2 ± 6.7	23.4 ± 7.8
HL activity (mU/ml)	377 ± 154[a]	378 ± 123	242 ± 134
LPL activity (mU/ml)	187 ± 61[b]	180 ± 63	225 ± 69
HL/LPL ratio	2.23 ± 1.15[a]	2.35 ± 1.20	1.22 ± 0.84
PLTP activity (nmol/ml/h)	6,540 ± 1,879[b]	6,328 ± 2,139	7,210 ± 1,762
PLTP mass (μg/ml)	5.26 ± 1.03[a]	5.29 ± 0.97	6.63 ± 1.32
CETP (nmol/ml/h)	39.1 ± 10.0[b]	37.0 ± 9.9	34.7 ± 7.0

Data are presented as means ± SD. CETP, cholesteryl ester transfer protein; HDL-C, high density lipoprotein cholesterol, LpA-I, lipoprotein A-I; LpA-I/A-II, lipoprotein A-I/A-II; PLTP, phospholipid transfer protein.

[a] Sex-, age-, and BMI-adjusted *P* value between affected low-HDL family members and control subjects was <0.001.

[b] Sex-, age-, and BMI-adjusted *P* value between affected low-HDL family members and control subjects was <0.050.

5.6% plasma apoA-I, respectively) and absolute amounts (16.2 ± 6.7 vs. 18.4 ± 7.5 mg apoA-I/dl, respectively) of preβ-HDL, although the differences were not statistically significant. Full descriptions of the clinical and biochemical characteristics of the low-HDL family members with CHD are given in Tables 1, 2.

The postheparin plasma activity of HL was significantly increased in the affected family members compared with the control subjects. In contrast, the activity of LPL was significantly decreased. The activity and mass concentration of PLTP was significantly lower in the affected family members than in the control subjects. In turn, CETP activity was significantly higher among the affected family members compared with the controls.

The protein/lipid composition of the HDL of the study subjects is presented in **Table 3**. The HDL_3 of the affected low-HDL family members contained relatively more TGs but less FC, cholesteryl esters, and phospholipids than that of the control subjects. The HDL_2 of the affected family members contained relatively more TGs but less FC and phospholipids than that of the control subjects.

The results of the correlation analysis between preβ-HDL concentration and clinical and biochemical parameters are presented in **Table 4**. Importantly, preβ-HDL correlated positively with TG in the affected family members ($r = 0.334$, $P = 0.006$) (**Fig. 1A**). There was a significant positive association also after the exclusion of subjects with CHD ($r = 0.408$, $P = 0.013$). Preβ-HDL concentration also showed a positive correlation with the levels of apoB ($r = 0.274$, $P = 0.025$) (Fig. 1B), LpA-I ($r = 0.383$, $P = 0.001$) (Fig. 1C), and TC ($r = 0.393$, $P = 0.001$). Higher preβ-HDL concentration was associated with the female gender (gender coded as male = 1, female = 2) in the affected low-HDL family members.

In the control subjects, preβ-HDL concentration showed a positive correlation with LpA-I as in the affected family members but did not show any statistically significant correlation with TG, TC, or apoB. Higher preβ-HDL concen-

TABLE 3. Protein/lipid composition of the HDL of the study subjects

Composition	Affected Low-HDL Family Members (n = 67; 55 men/12 women)	Control Subjects (n = 64; 42 men/22 women)
HDL_3-FC (% of HDL_3)	2.4 ± 0.5	2.7 ± 0.4[a]
HDL_3-CE (% of HDL_3)	17.9 ± 1.8	19.6 ± 2.3[a]
HDL_3-TG (% of HDL_3)	5.0 ± 1.4	2.9 ± 0.9[b]
HDL_3-PL (% of HDL_3)	20.9 ± 2.0	22.0 ± 2.8[a]
HDL_3-protein (% of HDL_3)	53.7 ± 2.9	52.7 ± 5.0
HDL_2-FC (% of HDL_2)	5.0 ± 1.8	5.9 ± 0.9[b]
HDL_2-CE (% of HDL_2)	20.8 ± 3.6	21.3 ± 2.9
HDL_2-TG (% of HDL_2)	8.9 ± 2.8	5.0 ± 1.7[b]
HDL_2-PL (% of HDL_2)	23.1 ± 2.3	27.0 ± 2.4[b]
HDL_2-protein (% of HDL_2)	42.2 ± 3.9	40.8 ± 3.3

Data are presented as means ± SD. CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid.

[a] Sex-, age-, and BMI-adjusted *P* value between affected low-HDL family members and control subjects was <0.050.

[b] Sex-, age-, and BMI-adjusted *P* value between affected low-HDL family members and control subjects was <0.001.

TABLE 4. Bivariate correlations between serum levels of absolute amounts of preβ-HDL and clinical and biochemical parameters in the study sample (n = 131)

Variable	Affected Low-HDL Family Members (n = 67)	Control Subjects (n = 64)
Age	0.007	0.308[a]
Gender	0.290[a]	−0.327[a]
Systolic blood pressure	0.117	0.248
BMI	0.101	0.066
W/H ratio	−0.050	0.340[a]
Glucose	−0.152	0.188
Insulin	0.217	0.056
HOMA IR	0.050	0.167
TC	0.393[a]	0.218
TG	0.334[a]	0.155
LDL	0.235	0.058
ApoB	0.274[a]	0.112
HDL-C	0.207	0.191
HDL_2-C	0.227	0.127
HDL_3-C	−0.067	0.184
VLDL-TG	0.239	0.168
ApoA-II	0.194	0.236
LpA-I	0.383[a]	0.273[a]
HL activity	−0.076	0.086
LPL activity	−0.114	0.162
HL/LPL ratio	0.050	−0.104
PLTP concentration	−0.177	−0.232
PLTP activity	0.027	0.056
CETP activity	0.140	−0.230

W/H ratio, waist/hip ratio. Gender was coded as male = 1, female = 2.

[a] $P < 0.050$.

tration was associated with the male gender in the control subjects, in contrast to the affected family members. Notably, preβ-HDL concentration correlated positively with W/H ratio in the control subjects.

Next, the relative contributions of different variables to preβ-HDL concentration were tested using stepwise regression analysis. In the affected family members, the variables that remained in the final model were TG, HDL-C, glucose, and gender, with the maximum adjusted multiple R^2 of 0.629 (**Table 5**). TG had by far the highest standardized coefficient in the final model, followed by HDL-C, glucose, and gender. In the control subjects, the remaining variables in the final model were gender ($r = -0.575$, $P < 0.001$), HDL-C ($r = 0.521$, $P < 0.001$), PLTP concentration ($r = -0.401$, $P = 0.004$), age ($r = 0.306$, $P = 0.017$), and insulin ($r = 0.274$, $P = 0.028$), with the maximum adjusted multiple R^2 of 0.775.

The results of the correlation analysis using HL activity as the dependent variable are shown in **Table 6** (only parameters with significant correlations are displayed). Higher HL activity was strongly associated with male gender in the affected low-HDL family members. In the affected family members, HL activity correlated positively with W/H ratio, TG, and VLDL-TG and correlated inversely with HDL-C and HDL_2-C. In the control subjects, HL activity showed an inverse correlation with HDL-C, HDL_2-C, and LpA-I. In addition, it correlated positively with insulin, HOMA IR, TC, LDL, VLDL-TG, and apoB but it did not correlate significantly with either W/H ratio or gender in the control subjects.

The correlation analysis was carried out also using PLTP concentration and activity as dependent variables. Among the affected low-HDL family members, PLTP activity showed positive correlation with PLTP mass ($r = 0.338$, $P < 0.05$), apoA-II ($r = 0.282$, $P < 0.05$), and LpA-I/A-II particles ($r = 0.344$, $P < 0.05$), whereas in control subjects, significant

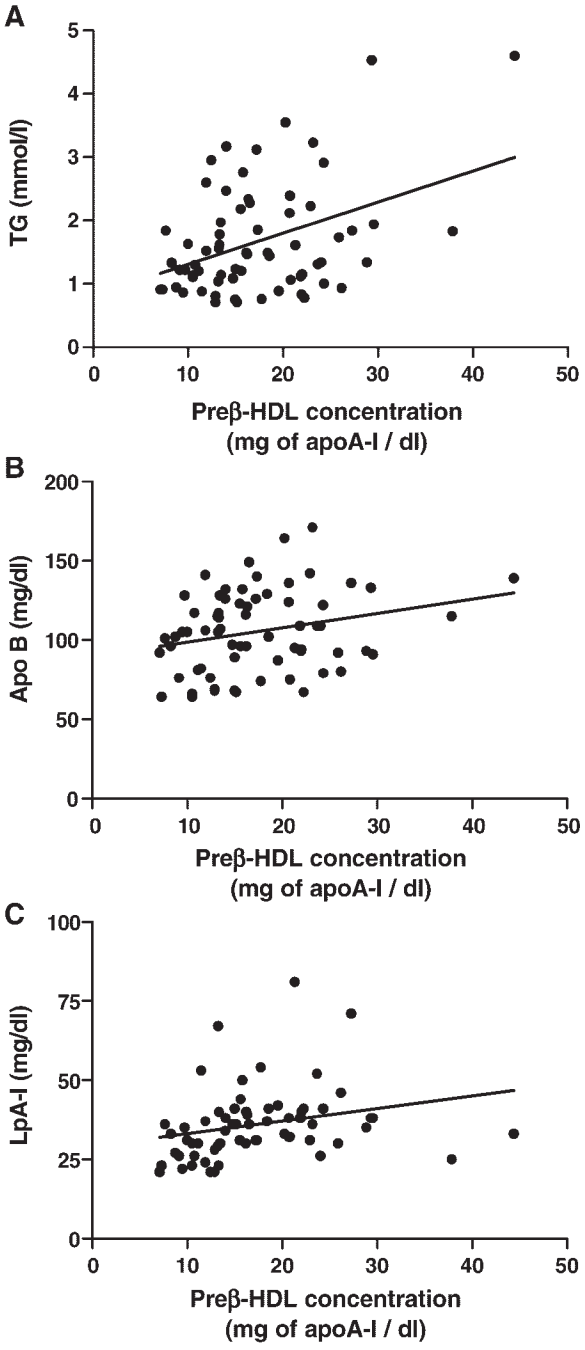

Fig. 1. Relationships between serum preβ-HDL concentration and triglycerides (TGs), apolipoprotein B (apoB), and lipoprotein A-I (LpA-I) in the affected low-HDL family members (n = 67). A: Correlation between serum preβ-HDL concentration and TGs ($r = 0.334$, $P = 0.006$). Preβ-HDL concentration and TG are $\log_{10}$ transformed. B: Correlation between serum preβ-HDL concentration and apoB ($r = 0.274$, $P = 0.025$). Preβ-HDL concentration is $\log_{10}$ transformed. C: Correlation between serum preβ-HDL concentration and LpA-I ($r = 0.383$, $P = 0.001$). Preβ-HDL concentration and LpA-I are $\log_{10}$ transformed.

TABLE 5. Multivariate regression analysis with preβ-HDL concentration and predictor variables in the affected low-HDL family members

Independent Variables	Standard Coefficient	SEM	*P*
TG	0.545	0.107	<0.001
HDL-C	0.378	0.296	0.009
Glucose	−0.282	0.025	0.016
Gender	0.255	0.054	0.049

The adjusted multiple R^2 of the model is 0.629. For data for the control sample, see text for details.

PLTP activity correlations were demonstrated only with PLTP mass and serum TC. On a protein level, there was a positive correlation between PLTP mass and HDL_3 among the affected family members. This correlation was not observed in control subjects. Instead, PLTP mass correlated significantly with total HDL-C ($r = 0.329$, $P < 0.05$) and large HDL_2 subpopulation ($r = 0.322$, $P < 0.05$) in the control group.

DISCUSSION

Low HDL-C is the most common familial lipoprotein abnormality in subjects with CHD before the age of 60 years (40–42). We report that the affected members of low-HDL families, in addition to the lower levels of "conventional" HDL parameters, had markedly reduced preβ-HDL concentrations. Preβ-HDL particles have a fundamental role in RCT, which is considered to be the major antiatherogenic function of HDL (1–3, 43). We examined preβ-HDL concentrations and major regulators of HDL metabolism in subjects with familial low HDL and in control subjects without any lipid abnormalities. A positive correlation was observed between preβ-HDL concentration and serum TG level in the affected low-HDL family members. Among them, serum TG level was also found to be the strongest predictor of preβ-HDL concentration. This correlation may be explained by the enhanced metabolic clearance of TG-enriched HDL by HL-mediated hydrolysis (44). The affected family members had higher serum TG levels and increased CETP activity, which leads to the TG enrichment of HDL. TG-enriched HDL is a preferred substrate for HL (17), and as a result, preβ-HDL particles are released.

When the association between preβ-HDL concentration and TG level was analyzed in subgroups, preβ-HDL concentration showed, surprisingly, a significant correlation with TG level only in family members free of CHD. The correlation pattern was also positive in family members who had CHD, although it was not significant ($r = 0.248$, $P = 0.178$). As discussed above, hypertriglyceridemia leads to decreased HDL as a result of enhanced clearance of TG-enriched HDL by HL. In this process, preβ-HDL formation is enhanced, which may maintain cellular cholesterol efflux as a defense mechanism against atherosclerosis. Therefore, the positive association between preβ-HDL and TG is shown in subjects with low HDL free of CHD. It could be hypothesized that in subjects with low HDL and CHD this defense mechanism had failed as they have developed CHD. One potential mechanism for this failure could be an enhanced catabolism of apoA-I and a loss of apoA-I from the body. Indeed, low-HDL family members free of CHD tended to have more preβ-HDL than family members with CHD.

In the present study, the actual amounts of preβ-HDL are increased compared with those reported in other studies. We propose that this difference is attributable to the use of different kinds of immunoassays. We used here an assay in which anti-apoA-I was mixed with the agarose gel, where the actual antibody-antigen reaction occurs. A totally different approach was used in previous studies, such as an ELISA with a specific anti-preβ1-recognizing monoclonal antibody (45, 46).

Currently, only limited data are available on the determinants of serum preβ-HDL levels in dyslipidemic individuals. Hypertriglyceridemic subjects have been shown to have increased levels of preβ-HDL (47). In a study of both normolipidemic and hypertriglyceridemic subjects by Fournier et al. (48), a positive correlation between the serum preβ-HDL concentration and TG level was shown. In contrast, O'Connor et al. (39) did not find a significant association between preβ-HDL and serum TGs in normolipidemic subjects. Also, in our healthy control subjects, no significant correlation was found between serum TGs and preβ-HDL concentration. The lack of correlation between preβ-HDL concentration and TG level in normolipidemic controls may be attributable to the limited range of plasma TG in normolipidemic subjects. This was suggested earlier by O'Connor et al. (39), who proposed that such an effect may be overshadowed by other determinants within the limited range of plasma TG in normolipidemic subjects. Another potential explanation is the pathway discussed above. Because normolipidemic subjects do not have hypertriglyceridemia, they do not have enhanced clearance of HDL by HL and thereby enhanced preβ-HDL production.

HL and CETP form preβ-HDL particles from larger HDL particles (17, 25). We observed that the activity of CETP and the postheparin plasma HL activity were higher

TABLE 6. Bivariate correlations between hepatic lipase activity and clinical and biochemical parameters in the study sample (n = 131)

Variable	Affected Low-HDL Family Members (n = 67)	Control Subjects (n = 64)
Gender	−0.461[a]	−0.064
W/H ratio	0.336[b]	0.143
Insulin	0.083	0.472[a]
HOMA IR	0.091	0.462[a]
TC	0.033	0.514[a]
TG	0.351[b]	0.230
VLDL-TG	0.339[b]	0.372[b]
LDL	−0.052	0.602[a]
ApoB	0.242	0.431[a]
HDL-C	−0.378[b]	−0.386[b]
HDL_2-C	−0.336[b]	−0.376[b]
LpA-I	−0.096	−0.331[b]

Gender was coded as male = 1, female = 2.
[a] $P < 0.001$.
[b] $P < 0.050$.

in the affected low-HDL family members than in the control subjects. On the basis of this result, it might be hypothesized that the affected low-HDL family members would produce higher levels of preβ-HDL as a result of the increased activities of HL and CETP. However, the affected low-HDL family members had significantly less preβ-HDL than did the control subjects. Therefore, the decrease in preβ-HDL levels in the affected low-HDL family members may not be attributable to an impaired formation of preβ-HDL but to an enhanced metabolic clearance of apoA-I. Lipid-free apoA-I can be catabolized via the kidney and liver if it is not remodeled to medium-sized HDL (49). It is known that cubilin acts as a receptor for the endocytosis of apoA-I in the kidney (50). The catabolism of lipid-free apoA-I in the kidney might be enhanced in subjects with familial low HDL and cause the decrease of preβ-HDL concentration.

HL activity showed an inverse association with HDL-C and HDL_2-C. This is in agreement with previous observations, which showed that in healthy men the TG-enriched HDL increased metabolic clearance as a result of enhanced HL-mediated hydrolysis (44). Moreover, these observations are in accordance with those made in subjects with familial combined hyperlipidemia, in whom the prolonged hypertriglyceridemia, together with increased HL activity, might be the central mechanisms for HDL lowering (51). Importantly, increased HL action has been shown to predominate also in insulin-resistant states, such as obesity and type 2 diabetes (52).

Preβ-HDL particles are also generated by the PLTP-mediated conversion of small HDL particles (14, 15, 17). Among the low-HDL family members, both PLTP activity and mass were significantly lower than among the control subjects. A recent study by Oka et al. (53) of hypoalphalipoproteinemic subjects demonstrated a reduction in PLTP mass with no significant differences in plasma PLTP activity. The data in these two studies are difficult to compare because of the different criteria for low HDL. The hypo (low)-HDL study population of Oka et al. (53) consisted of subjects with Tangier disease, LCAT deficiency, familial HDL deficiency, and apoA-I deficiency, whereas these dyslipidemias were excluded from our study material (54). In the present study, only a few correlations between PLTP and the measured parameters were evident. In control subjects, PLTP activity correlated with TC and showed a borderline association with LDL-C and apoB; no correlation was shown with HDL parameters. Similar observations were made in the recent Finnish Health 2000 Health Examination Survey (55) and previously by Murdoch and colleagues (56). The correlation pattern was different among the affected low-HDL family members: PLTP activity correlated with LpA-I/A-II particles and apoA-II. This suggests that among low-HDL subjects, active PLTP might be preferentially associated with HDL particles containing both major apolipoproteins of HDL. The correlation of PLTP mass with HDL-C and HDL_2-C supports the correlations observed for PTLP activity, as the major part of the circulating LpA-I/A-II particles are located in the larger HDL_2 subclass.

In the current study, none of the plasma factors discussed above (HL, PLTP, CETP) correlated with preβ-HDL concentration in the affected low-HDL family members. This lack of correlation between the proteins regulating HDL-C levels in the circulation and preβ-HDL concentration was rather surprising. On the other hand, it supports the idea that impaired preβ-HDL formation via HDL conversion may not be the mechanism behind the preβ-HDL decrease observed in the affected low-HDL family members. We suggest that the reason for their decreased preβ-HDL level may be the enhanced catabolism of lipid-free apoA-I.

The de novo formation of preβ-HDL particles from biosynthetically produced lipid-poor apoA-I particles is dependent on the efflux of intracellular cholesterol to the circulating apoA-I by the ABCA1 protein on the cell membranes. Serum preβ-HDL level correlates positively with cellular cholesterol efflux in nondiabetic, insulin-resistant subjects with increased plasma TG levels (57). A positive correlation has also been found between serum preβ-HDL level and cholesterol efflux in normolipidemic as well as hypertriglyceridemic and hyperlipidemic subjects (48). Higher plasma PLTP activity and enhanced preβ-HDL formation in insulin-resistant subjects with hypertriglyceridemia may maintain cellular cholesterol efflux as a defense mechanism against atherosclerosis. However, the data concerning the association between preβ-HDL concentration and cholesterol efflux in subjects with isolated low HDL-C and premature CHD are sparse, and we cannot exclude the possibility that efflux of cholesterol to lipid-poor preβ-HDL particles is reduced.

One potential limitation of this study is that we have not measured the activity of LCAT, which catalyzes the esterification of the nonesterified cholesterol on HDLs, increasing the size of HDL particles. However, LCAT activity was not altered in patients with familial HDL deficiency in a study by Marcil et al. (58). In fact, decreased LCAT activity leads to the accumulation of unesterified cholesterol in HDL particles and thereby potentially increases preβ-HDL levels.

The affected low-HDL family members had higher BMI, higher HOMA IR, and higher levels of insulin, glucose, TG, VLDL-TG, and apoB than did the control subjects. This finding is consistent with our previous studies of low-HDL families (27), suggesting that although recruited from families with low HDL and premature CHD, the affected family members present a complex phenotype resembling the metabolic syndrome. The underlying genetic cause predisposing to low HDL-C is still unknown in this study sample. No variations in the *ABCA1* region or other potential candidate genes, including the *APOA1C3A4* cluster or *APOA2*, have been observed in our low-HDL families. In turn, some linkage evidence was observed for the loci on chromosomes 8q, 16q, and 20q in a genome-wide scan (26). Interestingly, the regions on 16q and 20q have been linked to increased TGs, insulin resistance, and type 2 diabetes (59, 60).

To summarize, the present study showed that subjects with familial low HDL have a low concentration of serum

preβ-HDL. The strongest variable to predict preβ-HDL concentration was the serum TG level. Hypertriglyceridemia gives rise to TG enrichment of HDL particles, which in turn are rapidly hydrolyzed by HL. Thereby, preβ-HDL particles are released. One mechanism behind the increased cardiovascular risk in subjects with familial low HDL could be an impairment of the RCT pathway, in which preβ-HDL particles act as the primary acceptors of cellular cholesterol. Our results support this hypothesis, as the affected low-HDL family members had lower preβ-HDL concentrations than did the normolipidemic subjects. However, rather than having impaired formation of preβ-HDL, the subjects with familial low HDL may have enhanced metabolic clearance of the lipid-free apoA-I and, as a result, decreased preβ-HDL concentration.

The authors thank the family members and the control subjects for participating in this study. Jari Metso, Helinä Perttunen-Nio, Hannele Hilden, Tomi Silvennoinen, Ritva Marjanen, Ritva Keva, Ritva Nurmi, and Sari Nuutinen are thanked for their excellent technical assistance. The International HDL Research Awards (C.E.), the Sigrid Juselius Foundation (M.J., M-R.T.), the Finnish Foundation for Cardiovascular Research (M.J., M-R.T.), and the Helsinki University Central Hospital Research Foundation (M-R.T.) are acknowledged for their financial support.

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