# Determinants of low HDL levels in familial combined hyperlipidemia

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**Abstract In familial combined hyperlipidemia (FCHL), affected family members frequently have reduced levels of HDL cholesterol, in addition to elevated levels of total cholesterol and/or triglycerides (TGs). In the present study, we focused on those determinants that are important regulators of HDL cholesterol levels in FCHL, and measured postheparin plasma activities of hepatic lipase (HL), lipoprotein lipase, cholesterol ester transfer protein, and phospholipid transfer protein (PLTP) in 228 subjects from 49 FCHL families. In affected family members (n = 88), the levels of HDL cholesterol, $HDL_2$ cholesterol, $HDL_3$ cholesterol, and apolipoprotein A-I were lower than in unaffected family members (n = 88) or spouses (n = 52). The main change was the reduction of $HDL_2$ cholesterol by 25.4% in affected family members ($P < 0.001$ vs. unaffected family members; $P = 0.003$ vs. spouses). Affected family members had higher HL activity than unaffected family members ($P = 0.001$) or spouses ($P = 0.013$). PLTP activity was higher in affected than unaffected family members ($P = 0.025$). In univariate correlation analysis, a strong negative correlation was observed between HL activity and $HDL_2$ cholesterol ($r = -0.339$, $P < 0.001$). Multivariate regression analysis demonstrated that gender, HL activity, TG, and body mass index have independent contributions to $HDL_2$ cholesterol levels. We suggest that in FCHL, TG enrichment of HDL particles and enhanced HL activity lead to the reduction of HDL cholesterol and $HDL_2$ cholesterol.**—Soro, A., M. Jauhiainen, C. Ehnholm, and M-R. Taskinen. **Determinants of low HDL levels in familial combined hyperlipidemia. *J. Lipid Res.* 2003.** 44: **1536–1544.**

**Supplementary key words** $HDL_2$ cholesterol • hepatic lipase • triglycerides • phospholipid transfer protein • cholesteryl ester transfer protein • lipoprotein lipase • insulin resistance

Familial combined hyperlipidemia (FCHL) is a genetic disorder with a high prevalence in the population (1:100 individuals) (1, 2). Subjects affected with FCHL have a 10-fold higher risk for premature coronary artery disease (CAD) (1, 3). Regarding plasma lipid profiles, FCHL is characterized by elevated serum total cholesterol (TC), elevated triglycerides (TGs), or both, with affected individuals aggregating in families (1, 4). A consistent metabolic finding in FCHL patients is increased apolipoprotein B (apoB) concentration, although it is not clear whether an increased production or decreased clearance of apoB-containing lipoproteins predominates (5, 6). Moreover, FCHL patients often present features of insulin resistance syndrome: impaired glucose tolerance, abdominal obesity, and hyperinsulinemia (7–10). Low serum levels of HDL cholesterol are frequently found as a characteristic feature of the FCHL lipid profile (6). However, the mechanisms responsible for the reduction of HDL cholesterol and changes in HDL particle composition have not been established.

The ability of HDL to protect against the development of CAD has been well documented and, although the exact molecular mechanism(s) behind this finding is still unsolved, it is thought to be due to the role of HDL in reverse cholesterol transport (11, 12). The HDL in human plasma consists of several subpopulations of particles with distinct structure, function, and composition. This heterogeneity, which is the result of continuous remodeling of HDL by plasma factors, has important implications in terms of the cardioprotective functions of HDL. The regulation of HDL metabolism is achieved by the concerted action of a number of plasma and cellular factors. These include membrane proteins class B type I scavenger receptor (SR-BI) and ATP-binding cassette transporter-1; plasma proteins such as cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP), and lecithin:cholesterol acyltransferase; and endothelial-bound enzymes lipoprotein lipase (LPL), hepatic lipase (HL), and endothelial lipase (13–15). Studies in PLTP knockout mice have demonstrated that PLTP facilitates the transfer of postlipolytic surface remnants from VLDL and chylomicrons to HDL (16, 17). Thus, PLTP is an important factor in the maintenance of HDL levels. Another lipid transfer

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protein in plasma, CETP, modulates the transfer of cholesteryl esters from HDL to apoB-containing lipoproteins, resulting in TG enrichment of HDL (14). TG-enriched HDL particles are the preferred substrate for HL (13). HL-mediated hydrolysis of both TG and phospholipids on HDL particles transforms HDL into smaller, denser $HDL_3$ particles with a subsequent release of lipid-poor apoA-I particles (18–20). In fact, in vitro and animal studies have suggested that HL is the most important lipase affecting the catabolic rate of HDL in hypertriglyceridemic states (19, 21–23).

As the mechanisms responsible for the low HDL cholesterol levels in FCHL have not been resolved, we decided to study some of the determinants of HDL cholesterol, and therefore measured the postheparin plasma activities of HL and LPL, and the activities of CETP and PLTP in 228 subjects from 49 FCHL families.

## METHODS

### Study subjects

The Finnish FCHL families were recruited in Helsinki and Turku University Central Hospitals as part of the European Multicenter Study on Familial Dyslipidemias (EUFAM) (24). A total of 49 Finnish FCHL families, including 176 family members and 52 spouses, were studied. The EUFAM study protocol and inclusion criteria have been presented in detail previously (9, 24). The age-sex-specific percentile criteria used for classification of study subjects were derived from the population-based survey FINRISK (25, 26). The subjects were divided into three groups: affected family members (according to serum TC and/or TG exceeding the age-sex-specific 90th percentile), unaffected family members, and spouses. Any lipid-lowering medication was discontinued 4 weeks before collection of blood samples. Women under estrogen medication were not included in the study.

Each subject gave a written informed consent prior to participating in the study. The study design was approved by the Helsinki University Central Hospital ethics committee, and all the samples were collected in accordance with the Helsinki declaration.

### Biochemical analyses

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 (Hoffmann-La Roche, Basel, Switzerland) by enzymatic methods (Hoffmann-La Roche kits 0722138 and 0715166, respectively). 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 by differential electroimmunoassay (Sebia, Issy-les-Moulienaux, France) (27). The concentration of LpA-I-AII particles was calculated by subtracting the concentration of LpA-I from the total concentration of apoA-I in serum. LDL, HDL, $HDL_2$, and $HDL_3$ were separated by ultracentrifugation, as described (28). LDL particle size was determined using native gradient gel electrophoresis, as described in detail by Vakkilainen et al. (29). PLTP activity was measured using the radiometric assay described by Damen et al., with minor modifications (30–32). Plasma glucose concentration was analyzed by the glucose dehydrogenase method (Precision-G Blood Glucose Testing System, Medisense, Abbott Laboratories, Abbott Park, IL). Serum-free insulin concentration was 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 (uU/ml) × fasting glucose (mmol/l)/22.5 (33). CETP activity was measured by the method of Groener, Pelton, and Kostner (34). Postheparin plasma LPL and HL activities were measured as described (35).

### Demographic variables

Body weight and height were measured and body mass index (BMI) calculated as weight/height$^2$ (kg/m$^2$). Waist and hip circumference and waist-hip (w/h) ratio were recorded. Blood pressure was measured in the supine position, and hypertension was defined as systolic blood pressure (SBP) ⩾140 mmHg, diastolic blood pressure (DBP) ⩾90 mmHg, or self-reported use of antihypertensive medications. The smoking status of the subjects was categorized as current smokers or nonsmokers. Metabolic syndrome score was calculated for each study subject using the following determinants: abdominal obesity (waist circumference in men >102 cm and in women >88cm), TG >1.7mmol/l in both genders, HDL cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women, blood pressure ⩾130/85 mmHg, and fasting glucose ⩾6.1 mmol/l (36). The percentage of subjects presenting three or more determinants of the metabolic syndrome was calculated in each group (unaffected and affected FCHL family members and spouses).

### Statistical methods

Statistical comparisons of clinical and biomedical parameters were performed with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). Results are expressed as mean ± SD for continuous variables and as frequencies or percentages for categorical variables. Variables with skewed distribution were $\log_{10}$ transformed before the analyses, but the values in text, tables, and figures are presented as nontransformed. Continuous variables were compared between affected and unaffected family members, affected family members and spouses, and unaffected family members and spouses by general linear model, univariate ANOVA. $P < 0.050$ was considered significant (two-tailed). Because spouses were significantly older and had a different gender distribution than affected or unaffected family members, parameters in ANOVA were age and gender adjusted. In order to correct for the nonindependence of the subjects, a dummy variable indicating belonging to a certain family, having always a value of 1, 0, or −1, was created for each subject (37). The dummy variables were always included in ANOVA as covariates. The frequency distribution of the categorical variables in the three groups was compared by the $\chi^2$ test. The univariate correlation analysis was used to compare the relation between metabolic and demographic variables and $HDL_2$ cholesterol, both with and without gender adjustment. The family number indicators were included in the model as covariates in univariate correlation analysis. The family number indicators were not included in the model when the correlation analyses were performed in affected and unaffected family members separately. This was avoided because there were 35 families with less than two subjects in either the affected or unaffected groups. Stepwise regression analysis was performed to test the relative contribution of different covariates (independent variables) to $HDL_2$ cholesterol level (dependent variable). The covariates were selected on the basis of the univariate correlation analyses ($P < 0.050$, without gender adjustment). Insulin was excluded because it is a close correlate with HOMA IR, w/h ratio was excluded because it is in a close correlation with BMI, and LDL size was excluded because it is a close correlate with TG and apoB. Family number indicators and gender were always

forced into the model. As a next step, variables were removed from the model until the best fitting model with the maximum adjusted multiple $R^2$ was achieved.

## RESULTS

### Clinical characteristics

Clinical and biochemical characteristics of the study subjects are presented in **Table 1**. The gender distribution was similar in affected and unaffected groups of family members. Affected and unaffected family members did not differ from each other by age, but the mean age of the spouses group was higher. Hypertension was recorded in 15 affected family members, five unaffected family members, and 11 spouses. In the affected group, SBP and DBPs were significantly higher than in unaffected family members. Smoking habits were similar in the three groups. Affected family members had significantly higher BMIs than unaffected family members. The three groups had similar w/h ratio and fasting plasma glucose but, as expected, the fasting insulin level and HOMA IR were significantly higher in affected family members than in unaffected family members or spouses. These differences remained significant ($P < 0.050$) when adjusted for BMI. According to definition criteria (24), concentrations of TC, TG, LDL cholesterol, and apoB were significantly increased in affected family members. LDL particle size was significantly smaller in affected FCHL family members than in unaffected FCHL family members or spouses. The percentage of subjects presenting three or more determinants of the metabolic syndrome was significantly higher among affected family members (37.5%) than among unaffected family members (8.0%) or spouses (15.4%).

### Characteristics of HDL

The concentration of HDL cholesterol was significantly lower in affected family members (1.26 ± 0.35 mmol/l) than in unaffected family members (1.48 ± 0.36 mmol/l, $P < 0.001$) or spouses (1.38 ± 0.29 mmol/l, $P = 0.004$, Table 1.). Likewise, the levels of both $HDL_2$ cholesterol and $HDL_3$ cholesterol were lower in affected than unaffected family members or spouses (**Table 2**). The $HDL_2$ cholesterol level was 25.4% lower in affected family members compared with unaffected family members, and 14% lower compared with spouses ($P < 0.050$ for both). In addition, $HDL_2$ phospholipids were significantly reduced, whereas $HDL_2$ TGs and $HDL_3$ TGs were increased in affected family members. ApoA-I was lower in affected family members than in the unaffected group (140 ± 26 g/l vs. 146 ± 26 g/l, $P = 0.033$), but the levels of apoA-II, LpA-I, and LpAI-AII did not differ between the groups. Among male and female subjects, the affected men had significantly lower mean HDL cholesterol (1.06 ± 0.36

TABLE 1. Clinical and biochemical characteristics of study subjects

| | FCHL Affected [n = 88 (48 m/40 f)] | FCHL Unaffected [n = 88 (48 m/40 f)] | $P^b$ | Spouses [n = 52 (31 m/21 f)] | $P$ |
|---|---|---|---|---|---|
| Age (years) | 40.1 ± 11.4 | 37.7 ± 9.8 | n.s. | 51.1 ± 7.90 | <0.001[c,d] |
| Hypertensive subjects (%) | 17% | 6% | 0.018 | 21% | n.s.[c]; 0.005[d] |
| Smokers (%) | 45% | 36% | n.s. | 37% | n.s.[c,d] |
| BMI, kg/m² | 27.0 ± 4.0 | 25.4 ± 4.0 | 0.004 | 26.4 ± 4.5 | n.s.[c,d] |
| TC (mmol/l) | 6.27 ± 1.02 | 5.09 ± 0.79 | <0.001 | 5.48 ± 0.90 | <0.001[c]; n.s.[d] |
| TG (mmol/l) | 2.32 ± 1.39 | 1.05 ± 0.39 | <0.001 | 1.21 ± 0.43 | <0.001[c]; n.s.[d] |
| HDL cholesterol (mmol/l) | 1.26 ± 0.35 | 1.48 ± 0.36 | 0.001 | 1.38 ± 0.29 | 0.004[c]; n.s.[d] |
| LDL (mmol/l) | 4.00 ± 0.93 | 3.18 ± 0.78 | <0.001 | 3.59 ± 0.79 | <0.001[c]; n.s.[d] |
| ApoB (g/l) | 123 ± 27 | 86 ± 19 | <0.001 | 98 ± 20 | <0.001[c]; n.s.[d] |
| LDL size, (nm) | 25.7 ± 1.6 | 26.9 ± 1.2 | <0.001 | 26.7 ± 1.2 | <0.001[c]; n.s.[d] |
| Glucose (mmol/l) | 4.8 ± 0.7 | 4.7 ± 0.7 | n.s. | 5.0 ± 0.8 | n.s.[c,d] |
| Insulin (mU/l) | 10.1 ± 4.7 | 6.7 ± 3.6 | <0.001 | 7.1 ± 2.9 | <0.001[c]; n.s.[d] |
| HOMA IR | 2.22 ± 1.19 | 1.46 ± 0.92 | <0.001 | 1.64 ± 0.82 | <0.001[c]; n.s.[d] |
| Waist/hip ratio | 0.89 ± 0.10 | 0.87 ± 0.09 | n.s. | 0.91 ± 0.08 | n.s.[c,d] |
| Diastolic blood pressure | 84 ± 14 | 77 ± 11 | 0.001 | 81 ± 9 | 0.003[c]; n.s.[d] |
| Systolic blood pressure | 134 ± 18 | 125 ± 16 | <0.001 | 129 ± 16 | <0.001[c]; n.s.[d] |
| Pulse pressure | 50 ± 14 | 48 ± 10 | n.s. | 47 ± 12 | n.s. |
| Metabolic syndrome score[a] | | | | | |
| 3–5 Risk factors (%) | 37.5% | 8.0% | 0.001 | 15.4% | 0.005[c]; n.s.[d] |

Apo, apolipoprotein; BMI, body mass index; FCHL, familial combined hyperlipidemia; HOMA IR, homeostasis model assessment for insulin resistance; TC, total cholesterol; TG, triglyceride; LDL size, mean LDL particle size. Data are presented as mean ± SD, frequencies, or percentages. For statistical comparisons, general linear model, univariate analysis (with a dummy variable indicating belonging to a certain family included as a covariate) was used for continuous variables, and $\chi^2$-test was used for categorical variables. The variables with nonnormal distribution were $\log_{10}$ transformed.

[a] Metabolic syndrome score was calculated as indicated in the Methods.

[b] ANOVA for the comparisons between affected and unaffected family members, with adjustment for family number (dummy variables indicating the family numbers included in the analysis as covariates).

[c] ANOVA for the comparisons between spouses and affected family members, with adjustment for family number, age, and gender.

[d] ANOVA for the comparisons between spouses and unaffected family members, with adjustment for family number, age, and gender.

TABLE 2. HDL lipids and apolipoproteins

| | FCHL Affected (n = 88) | FCHL Unaffected (n = 88) | $P^a$ (ANCOVA) | Spouses (n = 52) | $P$ (ANCOVA) |
|---|---|---|---|---|---|
| $HDL_2$ cholesterol (mmol/l) | 0.63 ± 0.31 | 0.79 ± 0.33 | <0.001 | 0.72 ± 0.26 | 0.035[b]; n.s.[c] |
| $HDL_2$ TGs (mmol/l) | 0.11 ± 0.05 | 0.10 ± 0.05 | 0.011 | 0.10 ± 0.05 | n.s.[b,c] |
| $HDL_2$ phospholipids (mg/dl) | 35.8 ± 18.0 | 44.5 ± 20.4 | <0.001 | 40.9 ± 14.3 | n.s.[b,c] |
| $HDL_3$ cholesterol (mmol/l) | 0.63 ± 0.12 | 0.69 ± 0.13 | 0.002 | 0.66 ± 0.12 | n.s.[b,c] |
| $HDL_3$ TGs (mmol/l) | 0.11 ± 0.04 | 0.08 ± 0.04 | 0.002 | 0.08 ± 0.03 | 0.001[b]; n.s.[c] |
| $HDL_3$ phospholipids (mg/dl) | 42.0 ± 7.6 | 42.9 ± 9.5 | n.s. | 41.9 ± 8.1 | n.s.[b,c] |
| ApoA-I (g/l) | 140.0 ± 25.5 | 145.9 ± 25.8 | 0.033 | 143.4 ± 23.1 | n.s.[b,c] |
| ApoA-II (g/l) | 39.9 ± 6.8 | 40.3 ± 8.4 | n.s. | 37.9 ± 6.8 | n.s.[b,c] |
| LpA-I (mg/dl) | 52.0 ± 14.7 | 54.6 ± 16.6 | n.s. | 57.4 ± 15.2 | n.s.[b,c] |
| LpAI-AII (mg/dl) | 87.9 ± 17.0 | 91.3 ± 18.1 | n.s. | 86.0 ± 15.3 | n.s.[b,c] |

Lp, lipoprotein.

[a] $P$ values are presented for ANOVA between affected and unaffected family members, with family number adjustment.

[b] $P$ values are presented for ANOVA between affected family members and spouses, with adjustment for family number, age, and gender.

[c] $P$ values are presented for ANOVA between unaffected family members and spouses, with adjustment for family number, age, and gender.

mmol/l) than unaffected men (1.35 ± 0.46 mmol/l, $P <$ 0.001). Likewise, affected women had lower mean HDL cholesterol (1.39 ± 0.45 mmol/l) than unaffected women (1.54 ± 0.43 mmol/l), but this difference was not statistically significant.

### Activities of lipolytic enzymes and lipid transfer proteins

Serum activities of HL, PLTP, LPL, and CETP are shown in **Table 3**. HL activity was significantly higher in affected than in unaffected family members (356.5 mU/ml vs. 291.5 mU/ml; $P = 0.001$) or spouses (356.5 mU/ml vs. 281.8 mU/ml; $P = 0.013$). PLTP activity was also increased in affected family members as compared with unaffected family members ($P = 0.025$). The activities of LPL and CETP were comparable in the three groups. Total HDL cholesterol, $HDL_2$ cholesterol, $HDL_3$ cholesterol, and apoA-I all showed similar correlations with demographic variables (age, gender, BMI, and w/h ratio) and metabolic variables (TGs, TC, apoB, glucose, insulin, HOMA IR, CETP activity, PLTP activity, LPL activity, and HL activity) in correlation analyses. Because $HDL_2$ cholesterol revealed the strongest correlations, we present $HDL_2$ cholesterol correlation data (**Table 4**). $HDL_2$ cholesterol correlated strongly with gender ($r = 0.362$, $P < 0.001$). Therefore, partial correlations were also calculated using gender adjustment. A significant correlation existed between $HDL_2$ cholesterol and TG, apoB, LDL size, fasting insulin level, HOMA IR, w/h ratio, and BMI. Importantly, $HDL_2$ cholesterol was inversely correlated with HL activity ($r = -0.339$, $P < 0.001$ when adjusted for gender), as shown in **Fig. 1**.

The correlations between gender-adjusted $HDL_2$ cholesterol and HL activity were significant in the two groups analyzed separately (in affected family members, $r = -0.394$, $P < 0.001$, and in unaffected family members, $r = -0.416$, $P < 0.001$). LPL activity showed a positive correlation with $HDL_2$ cholesterol ($r = 0.142$, $P = 0.030$). PLTP or CETP activities did not have any significant correlation with $HDL_2$ cholesterol. Next, we used TG as a covariate in the correlation analyses. The association between $HDL_2$ cholesterol and HL activity remained statistically significant when adjusted for TG level and gender in both affected and unaffected family members, as well as in the whole group (in affected family members, $r =$ cholesterol 0.330, $P = 0.001$; in unaffected family members, $r = -0.362$, $P = 0.001$).

TABLE 3. Activities of lipolytic enzymes and lipid transfer proteins

| | FCHL Affected (n = 88) | FCHL Unaffected (n = 88) | $P^a$ (ANOVA) | Spouses (n = 52) | $P$ (ANOVA) |
|---|---|---|---|---|---|
| HL activity (mU/ml) | 356.5 ± 171.6 | 291.5 ± 138.1 | 0.001 | 281.8 ± 120.6 | 0.013[b]; n.s.[c] |
| PLTP activity (μmol/ml/h) | 4.66 ± 1.36 | 4.29 ± 1.34 | 0.025 | 4.49 ± 1.23 | n.s.[b,c] |
| LPL activity (mU/ml) | 211.7 ± 63.7 | 215.5 ± 68.8 | n.s. | 228.1 ± 67.2 | n.s.[b,c] |
| CETP activity (nmol/ml/h) | 21.1 ± 5.7 | 22.0 ± 6.8 | n.s. | 21.9 ± 5.7 | n.s.[b,c] |

CETP, cholesteryl ester transfer protein; HL, hepatic lipase; LPL, lipoprotein lipase; PLTP, phospholipid transfer protein.

[a] $P$ values are presented for ANOVA between affected and unaffected family members, with family number adjustment.

[b] $P$ values are presented for ANOVA between affected family members and spouses, with adjustment for family number, age, and gender.

[c] $P$ values are presented for ANOVA between unaffected family members and spouses, with adjustment for family number, age, and gender.

TABLE 4. Univariate correlations between $HDL_2$ cholesterol and potential predictors of $HDL_2$ cholesterol levels in FCHL family members (n = 176)

| | $HDL_2$ $r^a$ | $P^b$ Fam | $HDL_2$ $r^a$ | $P^c$ Fam + Gender |
|---|---|---|---|---|
| Age | 0.077 | n.s. | 0.100 | n.s. |
| Gender | 0.362 | <0.001 | | |
| TG | −0.378 | <0.001 | −0.301 | <0.001 |
| ApoB | −0.358 | <0.001 | −0.280 | <0.001 |
| LDL size | 0.508 | <0.001 | 0.424 | <0.001 |
| Insulin | −0.222 | 0.004 | −0.194 | 0.006 |
| HOMA IR | −0.206 | 0.007 | −0.162 | 0.022 |
| Waist/hip ratio | −0.340 | <0.001 | −0.167 | 0.052 |
| BMI | −0.321 | <0.001 | −0.288 | <0.001 |
| HL activity | −0.447 | <0.001 | −0.339 | <0.001 |
| LPL activity | 0.094 | n.s. | 0.142 | 0.030 |
| PLTP activity | −0.121 | n.s. | −0.096 | n.s. |
| CETP activity | 0.080 | n.s. | −0.053 | n.s. |
| TC | −0.069 | n.s. | −0.006 | n.s. |
| Glucose | −0.059 | n.s. | −0.033 | n.s. |

[a] $r$ = correlation coefficient.
[b] $P$ values with adjustment for family number [family number indicators (dummy variables) included in the analysis as covariates].
[c] $P$ values with adjustment for family number and gender.

The correlation analyses were also carried out using HOMA IR as dependent variable. HOMA IR showed a significant positive correlation with TC, TG, and apoB (data not shown). HOMA IR was inversely correlated with LDL size ($r = -0.274$, $P = 0.003$), LpA-I ($r = -0.268$, $P = 0.001$), apoA-I ($r = -0.271$, $P = 0.001$), HDL cholesterol ($r = -0.357$, $P < 0.001$), $HDL_2$ cholesterol ($r = -0.293$, $P = 0.002$), $HDL_3$ cholesterol ($r = -0.232$, $P = 0.007$), and LPL activity ($r = -0.247$, $P = 0.001$). Interestingly, HL activity showed a strong correlation with HOMA IR ($r = 0.309$, $P = 0.002$, using gender adjustment), as shown in **Fig. 2**. Similar correlations were observed when the two groups were analyzed separately (in affected family members, $r = 0.267$, $P = 0.020$, and in unaffected family members, $r = 0.469$, $P < 0.001$, respectively).

Next, the independent associations between $HDL_2$ cholesterol and other variables (gender, apoB, TG, HOMA IR, BMI, HL activity, and LPL activity) were tested using stepwise regression analysis. These variables were removed from the model until the best fitting model with the maximum adjusted multiple $R^2$ (0.548) was achieved. The variables that remained in the final model were gender, HL activity, TG, BMI, HOMA IR, and LPL activity (**Table 5**). Gender had by far the highest standardized coefficient in the final model ($r = 0.246$, $P < 0.001$), followed by HL activity ($r = -0.213$, $P = 0.008$), TG ($r = -0.194$, $P = 0.010$), and BMI ($r = -0.208$, $P = 0.016$). HOMA IR and LPL activity did not have independent contributions to $HDL_2$ cholesterol level in the multivariate analysis.

Finally, we carried out the correlation analysis between LDL particle size and lipid-modifying enzymes. A statistically significant association was observed between HL activity and age-adjusted LDL particle size, both in affected subjects analyzed in a separate group ($r = -0.320$, $P = 0.002$) as well as in the whole group ($r = -0.294$, $P = 0.001$, adjusted for age and family number indicator). However, the association between HL activity and LDL size did not remain statistically significant when adjusted for TG and age. LPL, CETP, and PLTP activities did not have significant effects on LDL particle size. Notably, the level of TG was the strongest predictor of age-adjusted LDL particle size in affected FCHL family members ($r = -0.652$, $P < 0.001$) and in the whole group ($r = -0.537$, $P < 0.001$).

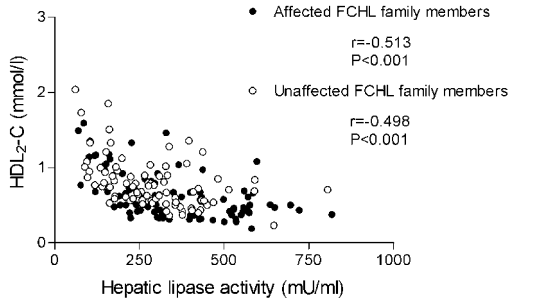


**Fig. 1.** Correlation between hepatic lipase (HL) activity and $HDL_2$ cholesterol in affected and unaffected familial combined hyperlipidemia (FCHL) family members. Open circles indicate unaffected FCHL family members (n = 88); solid circles indicate affected FCHL family members (n = 88). Correlation coefficients ($r$) and $P$ values are calculated without gender adjustment, separately in affected and unaffected family members, with $\log_{10}$-transformed $HDL_2$ cholesterol (in affected family members, $r = -0.513$, $P < 0.001$; in unaffected family members, $r = -0.498$, $P < 0.001$). The correlation results between $HDL_2$ cholesterol and HL activity were statistically significant with adjustment for gender: in affected family members, $r = -0.394$, $P < 0.001$; in unaffected family members $r = -0.416$, $P < 0.001$. Similar results were obtained when the affected and unaffected family members were analyzed in the same group; without adjustment for family number indicators or gender, $r = -0.515$, $P < 0.001$; with adjustment for family number and gender, $r = -0.339$, $P < 0.001$; see Table 4.

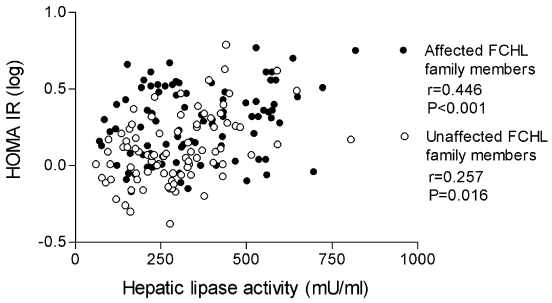


**Fig. 2.** Correlation between HL activity and $\log_{10}$-transformed homeostasis model assessment for insulin resistance [HOMA IR(log)] in affected and unaffected FCHL family members. Open circles indicate unaffected family members (n = 88), and solid circles indicate affected family members (n = 88). Correlation coefficients ($r$) and $P$ values are calculated without gender adjustment separately in affected and unaffected family members (in affected family members, $r = 0.257$, $P = 0.016$; in unaffected family members, $r = 0.446$, $P < 0.001$). Similar correlation results were observed with gender adjustment (in affected family members, $r = 0.267$, $P = 0.020$; in unaffected family members, $r = 0.469$, $P < 0.001$). Association between HOMA IR and HL activity was statistically significant also when affected and unaffected family members were analyzed in the same group ($r = 0.384$, $P < 0.001$, without gender or family number adjustment; $r = 0.309$, $P = 0.002$ with adjustment for family number indicator and gender).

TABLE 5. Multivariate regression analysis with $HDL_2$ cholesterol and predictor variables in FCHL family members (n = 176)

| Independent Variables | Standard Coefficient | Standard Error | *P* |
|---|---|---|---|
| Gender | 0.246 | 0.025 | <0.001 |
| TGs | −0.194 | 0.057 | 0.010 |
| HOMA IR | 0.112 | 0.063 | n.s. |
| BMI | −0.208 | 0.004 | 0.016 |
| HL activity | −0.214 | <0.001 | 0.008 |
| LPL activity | 0.090 | <0.001 | n.s. |

The adjusted multiple $R^2$ of the model is 0.548.

## DISCUSSION

In this large cohort of Finnish FCHL families, subjects affected by FCHL had decreased levels of HDL cholesterol, $HDL_2$ cholesterol, and $HDL_3$ cholesterol. Despite the pronounced decrease in $HDL_2$ cholesterol observed in affected family members, the levels of apoA-I and apoA-II, LpA-I, and LpAI-AII were not markedly altered, demonstrating alterations in the lipid-apolipoprotein ratio in HDL particles in affected subjects. Previous data on HDL lipid and apolipoprotein composition in FCHL have been inconsistent. In a study by Brunzell et al., the levels of total HDL cholesterol, apoA-I, and apoA-II in subjects affected by FCHL were comparable to those in the control subjects (38). In contrast, Ribalta et al. reported decreased levels of $HDL_2$ cholesterol in affected FCHL family members (39). In a recent, large follow-up study, neither HDL cholesterol nor apoA-I levels were significantly different among affected FCHL subjects and their matched controls (40).

What are the causative factors for the reduction of HDL cholesterol, particularly the reduction of cholesterol in $HDL_2$ subclass? Our results suggest that there are no major defects in the secretory or catabolic pathways of apoA-I or apoA-II, as the levels of apoA-I and A-II differed only slightly between affected and unaffected family members. In this context, it is important to recognize that HDL particles are continuously interconverted in the circulation. In FCHL, increased secretion of hepatic apoB-containing lipoproteins and delayed clearance of TG-rich particles result in a long residence time for TG-rich lipoproteins (TRLs) in the circulation (41). Thus, TGs may be efficiently transferred from TRL particles to HDL and exchanged for HDL cholesterol esters via the function of CETP (42). TG-enriched HDL particles are preferred substrates for hydrolysis by HL (18, 19, 43). An inverse relationship between HL activity and plasma HDL cholesterol has been reported in several studies (44–47). The HL-mediated lipolysis, a sum of phospholipid and TG hydrolysis, generates small, dense HDL particles as well as lipid-poor apoA-I particles, which are rapidly cleared from circulation, mainly via the kidneys (23, 48). Lamarche et al. have reported that the catabolism of TG-rich HDL is indeed enhanced in humans (49). Our results imply that a similar increase in catabolism of HDL particles due to enhanced HL activity takes place in FCHL, providing evidence for the role of HL-mediated hydrolysis as a major determinant of $HDL_2$ cholesterol. Taken together, our results suggest that the metabolic defect in FCHL leads to the prolonged hypertriglyceridemia and TG enrichment of the HDL particles. Eventually, increased lipolysis of TG-rich HDL particles by HL decreases the levels of HDL cholesterol, especially $HDL_2$ cholesterol.

We observed a positive association between HL activity and HOMA IR, an index of insulin resistance. As expected, affected family members had significantly higher fasting insulin levels and HOMA IR than unaffected family members when adjusted for BMI. Thus, our data confirm previous observations that FCHL patients share features of the insulin resistance syndrome, and emphasize low HDL as the consistent characteristic of the metabolic syndrome (8–10, 50). It remains to be established whether the increased HL activity is a general feature of the metabolic syndrome.

Besides having the role as a lipolytic enzyme, HL serves as a ligand that mediates the interaction between cell surface proteins and lipoproteins (51). In fact, HL has been shown to enhance the uptake of HDL cholesteryl esters via the SR-BI-mediated selective uptake (52). Data obtained from both association and linkage studies testing the relation between polymorphisms in the promoter region of the HL gene and HL activity or HDL cholesterol have been controversial. Some linkage-based studies have supported the linkage between the HL gene locus and FCHL trait, while others have not observed such an association (53–59). The HL gene locus has been linked to FCHL lipid abnormalities in Dutch FCHL families (53, 54). In addition, promoter polymorphisms in the HL gene have been shown to regulate insulin action and LDL TG content in FCHL patients (55) and CHD patients (47). In contrast, studies in Finnish FCHL families have not linked the HL locus to the FCHL trait (56, 57). A recent study in healthy women on the effects of the HL gene (LIPC) genotype demonstrated that subjects with increased HL activity have decreased levels of HDL cholesterol and $HDL_2$ cholesterol (60).

Though genes underlying the FCHL trait have not been identified, some promising linkage results for the FCHL loci have been reported (53, 56, 57, 61–63). The families in the current study were also included in the original Finnish genome-wide scan for FCHL (57). These genome scan data have been reanalyzed for the 10th percentile HDL cholesterol trait, and three chromosomal regions on chromosomes 8q, 16q, and 20q have provided some evidence for linkage (58). Recently, evidence for low HDL cholesterol loci was obtained on chromosome 16q24.1 in combined analysis of Dutch (63) and Finnish FCHL genome scans (59). A potential candidate gene in this 16q region is the *FOXC2* gene, which, in fact, is associated with obesity, hypertriglyceridemia, and insulin resistance (64). Furthermore, sequence analysis on the *FOXC2* gene showed an association between *FOXC2* allelic variants and the TG trait, suggesting that *FOXC2* may regulate the TG and/or HDL cholesterol levels in the families with FCHL (63).

A previous study on Finnish men showed that PLTP activity associates with TG, BMI, and LDL cholesterol, but

not with HDL cholesterol (31). In contrast, a study of healthy premenopausal women showed a positive association between PLTP activity and BMI, HDL cholesterol, and LDL cholesterol (65). Likewise, a positive association existed between PLTP activity and HDL cholesterol, $HDL_3$ cholesterol, apoA-I, and LpA-I in patients with CAD and low HDL cholesterol (66). In our study, affected FCHL family members showed increased PLTP activity. However, PLTP did not associate with either HDL parameters or HOMA IR. Therefore, evidence for a direct role of PLTP in affecting HDL cholesterol metabolism in FCHL patients is only suggestive. TG enrichment of HDL particles enhances the PLTP-mediated conversion of HDL to larger and smaller particles (67). Thus, the increased PLTP activity observed in affected FCHL family members can be explained by hypertriglyceridemia. These data imply that elevated TGs have an important modulating role for PLTP function via an unknown mechanism.

CETP converts large HDL particles into smaller sized HDL particles in concerted action with HL (13, 68). In previous studies, plasma CETP level was shown to be increased in patients with Type II diabetes, as well as in subjects with FCHL, hypercholesterolemia, and combined hyperlipidemia (69–71). In contrast, in our study sample, the activity levels of CETP were comparable among affected and unaffected family members, and serum HDL cholesterol and $HDL_2$ cholesterol were not correlated with CETP activity. Our data are in line with the study by Murakami et al., who found no differences in plasma CETP concentrations among hyperlipidemic patient groups and normolipidemic subjects (72). Therefore, we suggest that CETP activity does not independently contribute to HDL cholesterol level or HDL particle remodeling.

Reduced LPL activity results in an FCHL-like lipoprotein pattern, i.e., elevated levels of TC and TGs and decreased HDL cholesterol (73, 74). In the present study, no significant differences were observed in LPL activity among affected and unaffected family members. Likewise, LPL activity did not show any independent contribution to the $HDL_2$ cholesterol level in the correlation analyses. A study of adipose tissue LPL activity in FCHL showed similar results, with no difference between the FCHL patients and normolipidemic control subjects (75). Taken together, abnormal function of LPL is probably not a central metabolic defect in FCHL, despite the important role of LPL in TG and fatty acid metabolism.

Small, dense LDL particles, i.e., LDL subclass pattern B, are associated with hypertriglyceridemia, increased apoB, and decreased HDL cholesterol in FCHL patients (76–78). In the present study, HL activity correlated significantly with age-adjusted LDL particle size. However, the concentration of TG was the strongest predictor of age-adjusted LDL particle size, confirming our previous results (29). Long residence time of TRLs favors core lipid exchange between LDL and TRLs. TGs in LDL, like in HDL, are a good substrate for HL. Taken together, there seems to be a symmetry of the mechanisms leading to the generation of small LDL size and to lowering of the amount of HDL particles.

In conclusion, our study demonstrates that subjects affected by FCHL have decreased levels of HDL cholesterol, the major cholesterol reduction being in the $HDL_2$ subclass. Our results are consistent with the concept that in hypertriglyceridemia, HDL particles are TG-enriched, and HDL particle distribution is skewed toward smaller HDL subclasses. These data show for the first time that enhanced HL activity contributes significantly to the HDL reduction in FCHL dyslipidemia. Future studies are required to determine whether TG enrichment of HDL perverts HDL's antiatherogenic potential by impairing the process of cholesterol efflux.

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