Triglyceride-Rich HDL$_3$ from Patients with Familial Hypercholesterolemia Are Less Able to Inhibit Cytokine Release or to Promote Cholesterol Efflux$^1$

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ABSTRACT Familial hypercholesterolemia (FH) is associated with heterogeneity of the onset and severity of coronary heart disease (CHD). In this study, we investigated different low-grade proinflammatory markers and the atheroprotective function of the HDL$_3$ subfraction in FH-patients ($n = 13$) with identical LDL-receptor mutations and in age- and sex-matched healthy controls ($n = 11$). Compared with healthy controls, FH-patients had greater gene expressions of the proatherogenic mediators TNF-$\alpha$ and IL-8 in circulating peripheral blood mononuclear cells. In addition, they had a higher serum concentration of intercellular adhesion molecule-1 (ICAM-1) and a lower net antioxidant capacity. FH-derived HDL$_3$ with a high level of triglycerides had a reduced capacity to inhibit the release of IL-8 from TNF-$\alpha$-stimulated human umbilical vein endothelial cells (HUVEC) [1.864 mg/L (1.461–2.208 mg/L) vs. 1.466 mg/L (1.225–1.643 mg/L); $P < 0.05$; median (range)], and a reduced capacity to promote cholesterol efflux from lipid-loaded macrophages [12% (12–14%) vs. 15% (14–18%); $P < 0.05$; median (range)] compared with HDL$_3$ with a lower triglyceride content. Notably, the degree of inhibition of IL-8 release from HUVEC by HDL$_3$ was correlated with the ability of HDL$_3$ to promote cholesterol efflux ($r = -0.80, P = 0.03$). In conclusion, compared with healthy controls, FH-patients are characterized by higher levels of low-grade proinflammatory markers, and FH-derived HDL$_3$ with high triglyceride content may be more proatherogenic. These triglyceride-rich HDL$_3$ might be partly responsible for the phenotypic variation among FH-patients with identical LDL-receptor mutations. J. Nutr. 136: 877–881, 2006.

KEY WORDS: • familial hypercholesterolemia; high density lipoprotein • coronary heart disease • cytokines • cholesterol efflux

Familial hypercholesterolemia (FH)$^1$ is caused by a mutation in the gene coding for the LDL-receptor (1). Heterozygote FH-patients are characterized by elevated concentration of serum cholesterol (7–15 mmol/L), premature atherosclerosis, and coronary heart disease (CHD) (2). HDL cholesterol is inversely correlated with CHD (3,4); the major underlying protective mechanism is suggested to be reverse cholesterol transport. Other protective mechanisms of HDL unrelated to the plasma lipid transport, such as anti-inflammatory properties, are also considered to play an important role in preventing CHD (5,6), and it was suggested that the antiatherogenic function of HDL is a better marker than HDL cholesterol concentration (7) to distinguish between patients and healthy individuals. HDL can be differentiated into 2 major subfractions, HDL$_2$ and HDL$_3$, which each have antiatherogenic properties. HDL$_3$ is considered to be the major fraction, representing up to 90% of the total HDL (8). It has a greater capacity to accept cholesterol and has greater anti-inflammatory and antioxidant capacities than HDL$_2$ (9,10). Furthermore, experimental and clinical studies demonstrated that both hypercholesterolemia and hypertriglyceridemia promote endothelial dysfunction (11) and that HDL and/or reconstituted HDL improve endothelial functions (12). A proinflammatory milieu was shown to promote qualitative changes of the HDL particle by modifying the size and the composition of the particle (13); it is therefore of interest to study whether differences in composition attenuate the HDL function among subjects with increased atherosclerosis and elevated risk of CHD, such as FH-patients. The heterogeneity of the onset and severity of CHD varies among FH-patients with identical mutations (14,15). In the present study we searched for additional atherogenic factors that could at least in part explain the phenotypic variation among FH-patients with identical LDL-receptor mutations. Therefore, the aims of the study were to investigate (1) whether FH-patients were...
characterized by increased levels of low-grade proinflammatory markers compared with healthy controls, and (2) whether the composition of the HDL₁ subfraction isolated from FH-patients influenced the function of the HDL₁ particle with respect to inflammation and cholesterol efflux.

MATERIALS AND METHODS

Subjects. This is a subset of previously reported studies with FH-patients recruited consecutively from the Lipid Clinic at Rikshospitalet University Hospital, Norway (16,17). Those FH-patients from whom both peripheral blood mononuclear cells (PBMC) and HDL₁ were available (n = 13), were included in the present study. All FH-patients were diagnosed as heterozygous FH-Elevenum, by DNA test (18). Two FH-patients had diagnosed premature coronary artery disease (CAD), defined as myocardial infarction at an age ≤50 y, CAD was confirmed by coronary angiography showing ≥1 vessel disease (>75% narrowing of luminal diameter). The healthy control subjects were 11 age- and sex-matched volunteers with no history of CAD, diabetes, hypertension, or other acute or chronic illnesses. Written informed consent for participation was obtained from each participant; the study was approved by the regional Committee of Medical Ethics and complies with the Declaration of Helsinki. Before blood sampling, all FH-patients had a 4-wk wash-out period without statin treatment. Blood from venipuncture was collected after an overnight fast and drawn into pyrogen-free tubes (Becton Dickinson) without additives (serum) and with EDTA or heparin as anticoagulant (plasma). The serum tubes were allowed to clot for 30 min before centrifugation at 1000 × g for 15 min (serum), or centrifuged within 15 min at 1000 × g for 4°C for 15 min (plasma). Plasma for HDL isolation was diluted in sucrose (0.6%), and both serum and plasma samples were frozen (−80°C) until further analysis. PBMC were isolated by heparinized blood using isopaque-Ficoll (Lymphoprep, Nycomed Pharma AS) and gradient centrifuged within 45 min. PBMC pellets were immediately frozen and stored (−80°C).

Isolation of lipoproteins and oxidation of LDL. LDL (d 1.019–1.063 kg/L) from a healthy control and HDL₁ (d 1.12–1.21 kg/L) from FH-patients and matched control subjects were isolated from plasma by sequential ultracentrifugation using a TI 80 rotor (Beckman Optima LE-80K ultracentrifuge) as previously described (17). Total protein concentration was determined by the bichoninic acid protein assay (Pierce) using bovine serum albumin (BSA) as a standard. Interassay and intra-assay CV of protein measurement was 5.7 and 3.8%, respectively. LDL was oxidized in the presence of Cu²⁺ (10 µmol/L) for 24 h as previously described (19). The oxidation was terminated by refrigeration and the addition of EDTA (100 µmol/L final concentration).

Cell cultures. Primary human umbilical vein endothelial cells (HUVEC) were isolated and cultured in endothelial cell growth medium as previously described (17). The cells were harvested in 0.1% Triton X-100 lysis buffer, and culture lystate (supernatant) was collected after centrifugation (10,000 × g; 5 min at 4°C; Biofuge, Heraeus) and stored (−80°C) until further analyses. Human monocytic leukemia cells (THP-1 cells, art. no TIB-202) were purchased from the American Type Culture Collection and seeded into 24-well plates (750,000 cells/well) cultured in RPMI [1640 medium containing glutamine (4 mM/L), penicillin (20 KIU/L), streptomycin (20 mg/L) and bovine fetal calf serum (FCS; 10% final concentration; Gibco BRL)]. Cells were differentiated into macrophages by incubation for 72 h with phorbol 12-myristate 13-acetate (PMA, 100 nmol/L; Sigma Chemical). Cell viability of HUVEC and THP-1 macrophages was assessed using phase contrast microscopy to evaluate the morphology before and after the various experiments.

Oxygen radical absorbance capacity (ORAC). Antioxidant scavenging capacity was determined using the improved ORAC-fluorescein (FL) method for blood plasma (20), with modifications for using a fluorescence plate reader (21). The measurements were carried out on a Wallac 1420 Victor 96-well plate reader (PerkinElmer Life and Analytical Sciences, Wallac Oy) using the 485-nm excitation and the 535-nm emission filters. FL was used as the fluorescent substrate (70 nmol/L final concentration), 2,2'-azobis (2-amidinopropane) dihydrochloride as a source for the peroxyl radicals (12 nmol/L final concentration). Plasma samples were automatically diluted with 75 nmol/L potassium phosphate buffer, pH 7.4 by Biomek 2000 Workstation (Beckman Coulter) and added to a black 96-well microplate (96F, Nunc). The extent of protection against the peroxyl radicals by the antioxidants in the plasma sample was measured every 2 min until the fluorescence was <5% of the initial reading. Total antioxidant capacity was calculated using differences of areas under the FL decay curves between the blank and a sample. Trollox® (a vitamin E analog) was used as a control standard, and the degree of decolorization was related to that introduced by Trollox and presented as micromole Trollox equivalents (TE)/L plasma (µmol TE/L) and as total antioxidant capacity/total lipids (total serum cholesterol + triglycerides), representing µmol TE equivalents/µmol total lipids.

Real-time quantitative RT-PCR. Total RNA was extracted from PBMC using MagNa Pure LC RNA kit 1 (Roche Diagnostics). Conversion of mRNA to single-stranded cDNA was performed by a High Capacity cDNA Archive Kit (Applied Biosystems). To quantify the mRNA levels, qRT-PCR by SYBR Green I Dye Chemistry kit (Applied Biosystems) was used and the relative gene expression was calculated by standardizing the amount with β-actin as a housekeeping gene. Primers for TNF-α (forward primer [FP]: 5’-TCTGCTTCA-GTTGTCTGACCAG-3’ and reverse primer [RP]: 3’-AGGCACACA-GGCGGTGACAT-3’), macrophage inflammatory protein (MIP)-1α (FP: 5’-CAGCCACCAATGGGCTGACA-3’ and RP: 5’-CAACAGTTTG- GGAGGAAACCTT-3’), IL-8 (FP: 5’-GCCAACACAGGAATATT-GTGAAGGTT-3’ and RP: 5’-CCTCTCACCAAGCCTTCTTCC-3’ and β-actin (FP: 5’-TCCTCACATGTGAGAGCAT-3’ and RP: 5’-AGGCCACAGGGCGGTGACAT-3’) were designed using the Primer Express software version 1.5 (Applied Biosystems).

Cholesterol efflux. THP-1 macrophages were lipid loaded by incubation with oxidized LDL (20 mg/L) in growth medium (RPMI 1640 medium with FCS) and [0.5 mCi/L (18.5 mBq/L)] [3H]-Cholesterol; American Radiolabel Chemicals] dissolved in ethanol, was added. After 24 h, radiolabeled media were removed and the foam cells were washed twice with 0.2% BSA (wt:v) in RPMI. HDL₃ (final concentrations 50 mg/L) were then incubated for 3 h in RPMI 1640 medium (without FCS). Thereafter, the cell medium was collected and the cells were harvested in 0.2 mol/L NaOH. The radioactivity was measured by liquid scintillation counting using TRI-CARB 2300 TR Scintillation Counter (Packard). Data are presented as fractional (%) cholesterol efflux calculated as [dpm (media)/dpm (media + cell-associated)] × 100.

Biochemical analysis and enzyme immunoassays. Plasma triglycerides and plasma total-, HDL-, and LDL-cholesterol, plasma apolipoprotein A1, and plasma apolipoprotein B were measured using enzymatic colorimetric tests (Roche Diagnostics; inter- and intra-assay CV were <3% for all assays). Plasma homocysteine was measured by the Abbott homocysteine assay (Abbott Laboratories), a fully automated fluorescence polarization immunoassay method. Plasma total homocysteine concentrations were calculated by the Abbott IMx Immunooassay Analyzer using a machine-stored calibration curve. C-reactive protein (CRP) was measured in serum using a high-sensitivity particle enhanced immunoturbidimetric assay on a Modular platform (Roche Diagnostics). Serum concentrations of intercellular adhesion molecule-1 (ICAM-1) and MIP-1β, and IL-8 in culture medium were measured by enzyme immunoassays purchased from R & D systems. The total cholesterol, triglyceride, and phospholipid content of the HDL₃ was determined by commercially available enzymatic kit methods (Bayer and BioMérieux, respectively) in a Technicon RA-1000 system (Technicon Instruments). The HDL₃ composition is presented as mmol/ml protein.

Statistical analysis. Statistical significance between 2 groups was determined using Student’s t test when data were normally distributed and the Mann-Whitney U-test when not normally distributed. Data are expressed as means ± SD or medians (range), respectively. Spearman’s rank order correlations were determined with the entire study population included in each analysis. Differences were considered significant at P < 0.05 for all analyses. SPSS 11.0 for Windows was used for statistical analysis.
RESULTS

Characterizations of FH-patients and healthy controls

The main clinical and biological characteristics of FH-patients (n = 13) and age- and sex-matched healthy control subjects (n = 11) are presented in Table 1. Plasma concentrations of total cholesterol, LDL-cholesterol, triglycerides, and apolipoprotein B, and BMI were significantly higher among FH-patients than in healthy controls. Plasma HDL-cholesterol, apolipoprotein A-I, CRP, lipoprotein (a), and homocysteine did not differ between the groups.

**FH-patients vs. healthy control subjects**

**Gene expression of proinflammatory markers in PBMC.** FH-patients had higher mRNA levels of TNF-α (P = 0.02) and IL-8 (P = 0.03) (Fig. 1A,B) compared with healthy controls, and tended to have more MIP-1β mRNA (P = 0.06) (Fig. 1C). Plasma total and LDL cholesterol were correlated with the mRNA levels of TNF-α (r = 0.64, P = 0.001; r = 0.59, P = 0.01, respectively) and IL-8 (r = 0.51, P = 0.02; r = 0.51, P = 0.02, respectively).

**Proinflammatory markers in serum.** Compared with healthy control subjects, FH-patients had elevated serum ICAM-1 (P = 0.03), and serum MIP-1β tended to be higher (P = 0.06, Table 2). Serum concentrations of ICAM-1 and MIP-1β were correlated with total cholesterol (r = 0.48, P = 0.02; r = 0.48, P = 0.02, respectively), but not with LDL cholesterol.

**Total antioxidant capacity in serum.** Contrary to our expectations, the total antioxidant capacity did not differ between FH-patients and healthy control subjects (Table 2). However, the total antioxidant capacity:total lipids ratio was lower in FH-patients than in controls, suggesting a lower net antioxidant capacity (P = 0.001, Table 2).

**Phenotypic variations in FH-patients**

**Composition of HDL3 subfraction.** High serum levels of triglycerides (>1.7 mmol/L) are associated with increased risk of CVD (22); therefore, FH-patients were divided in 2 groups defined as FH-patients with serum triglycerides > or < 1.7 mmol/L. In all other variables measured (Table 1), the 2 groups of FH-patients were similar. FH-patients with serum triglycerides >1.7 mmol/mg protein had HDL3 with a higher level of triglycerides [0.07 mmol/L (0.06–0.10 mmol/mg protein) vs. 0.04 mmol/mg protein (0.03–0.08 mmol/mg protein); P = 0.01; median (range)] and a lower level of cholesterol [0.61 mmol/mg protein (0.40–0.63 mmol/mg protein) vs. 0.75 mmol/mg protein (0.54–0.95 mmol/mg protein); P = 0.02; median (range)] than FH-patients with serum triglycerides <1.7 mmol/L. The concentration of phospholipids did not differ between the 2 groups (data not shown). In addition, HDL3-triglycerides and serum triglycerides were correlated (r = 0.88, P < 0.001), and HDL3-triglycerides and HDL3-cholesterol were inversely correlated (r = −0.56, P = 0.05).

**Effect of triglyceride (TG)-rich HDL3 on secretion of IL-8 from TNF-α-stimulated HUVEC.** FH-patients were ranked according to the high or low content of triglycerides in their HDL3 (highTG-HDL3 and lowTG-HDL3, n = 6 and n = 7, respectively), defined as either above or below the median (0.05 mmol/mg total protein) of the triglyceride content in the HDL3. The highTG-HDL3 group was less able to inhibit the release of IL-8 from HUVEC than the lowTG-HDL3 group [1.864 mg/L (1.461–2.208 mg/L) vs. 1.466 mg/L (1.225–1.643 mg/L); P < 0.05; median (range), Fig. 2A].

Table 1

<table>
<thead>
<tr>
<th>Baseline characteristics of the FH patients and the healthy controls</th>
<th>FH-patients (n = 13)</th>
<th>Control subjects (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women/men, n/n</td>
<td>7/6</td>
<td>6/5</td>
</tr>
<tr>
<td>Age, y</td>
<td>48.3 ± 14.6</td>
<td>46.3 ± 13.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.3 ± 4.7*</td>
<td>22.5 ± 1.9</td>
</tr>
<tr>
<td>Premature coronary artery disease, n</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Tendon xanthomas/xanthelasmas, n</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Coronary arcus present, n</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Antihypertensive treatment, n</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Current smokers, n</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>9.2 ± 4.4**</td>
<td>4.7 ± 1.0</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mmol/L</td>
<td>7.1 ± 1.7**</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>Plasma HDL cholesterol, mmol/L</td>
<td>1.2 ± 0.3</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td>1.7 (0.7–8.8)*</td>
<td>0.7 (0.4–3.1)</td>
</tr>
<tr>
<td>Plasma lipoprotein (a), mg/L</td>
<td>161 (&lt;96–787)</td>
<td>99 (&lt;96–398)</td>
</tr>
<tr>
<td>Plasma apolipoprotein B, g/L</td>
<td>1.9 ± 0.4**</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Plasma apolipoprotein A-I, g/L</td>
<td>1.3 ± 0.2</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Plasma C-reactive protein, mg/L</td>
<td>2.3 ± 3.5</td>
<td>1.7 ± 1.7</td>
</tr>
<tr>
<td>Plasma homocysteine, μmol/L</td>
<td>8 (6–14)</td>
<td>10 (7–13)</td>
</tr>
</tbody>
</table>

*Values are means ± SD or median (range). Asterisks indicate different from controls: *P < 0.05, **P < 0.001.

FIGURE 1 Gene expression of TNF-α (A), IL-8 (B), and MIP-1β (C), relative to β-actin expression. Values are means ± SEM, n = 13 (FH) and n = 9 (controls). Means differed at the probabilities shown.
Effect of TG-rich HDL₃ on cholesterol efflux. Previous studies demonstrated that anti-inflammatory HDL particles promote cholesterol efflux more than proinflammatory HDL particles (23). In a random subgroup of FH-patients (n = 9), highTG-HDL₃ (n = 3) had a reduced capacity to induce cholesterol efflux from macrophages compared with lowTG-HDL₃ (n = 6) [12% (12–14%) vs. 15% (14–18%); P < 0.05; median (range); Fig. 2B]. Notably, the degree of inhibition of IL-8 from HUVEC by HDL₃ was inversely correlated with the ability of HDL₃ to promote cholesterol efflux (r = −0.80, P = 0.03, n = 7), whereas the triglyceride content of the HDL₃ subfraction tended to be correlated with the cholesterol efflux (r = −0.64, P = 0.07, n = 9).

DISCUSSION

The following observations were made in this study: 1) Compared with healthy controls, FH-patients had increased gene expression of proatherogenic cytokines in freshly isolated PBMC, elevated serum concentration of circulating proinflammatory markers, and a lower net antioxidant capacity. In addition, the proinflammatory markers and total and LDL cholesterol concentrations were correlated (2). FH-derived TG-rich HDL₃ were less able to inhibit TNF-α-induced release of IL-8 from HUVEC and to promote cholesterol efflux from lipid-loaded macrophages compared with FH-derived HDL₁ with a lower triglyceride content. Moreover, there was a strong inverse correlation between the triglyceride content of the HDL₃ and the ability to promote cholesterol efflux; most notable, however, was the strong correlation between the capacity of HDL₃ to inhibit release of IL-8 from TNF-α-stimulated HUVEC and to promote cholesterol efflux from lipid-loaded macrophages.

In this study, we demonstrated that FH-patients are characterized by increased levels of proinflammatory markers that are strongly related to the level of total and LDL cholesterol. Our findings support and extend our previously reported data demonstrating enhanced chemokine response from monocytes in FH-patients (16).

Antioxidants may reduce systemic inflammation, reverse endothelial dysfunction, and protect circulating lipids from oxidation. We compared the antioxidant capacity in FH-patients and healthy controls given the total circulatory lipid content and showed that there was an unfavorable antioxidant ratio in FH-patients, suggesting an increased demand for antioxidants in these patients. This may indicate that lipoproteins in FH-patients are more susceptible to oxidative modifications.

Furthermore, we showed here that there was a significantly higher level of serum triglycerides in the HDL₃ from FH-patients with serum triglycerides >1.7 mmol/L compared with FH-patients with serum triglycerides <1.7 mmol/L. This is consistent with other reports suggesting triglyceride enrichment of HDL particles secondary to hypertriglyceridemia (24). In addition, we recently reported an increased triglyceride content in HDL₃ derived from FH-patients with premature CAD (17). In the present study we extend these data by demonstrating a decreased ability of FH-derived TG-rich HDL₃ to inhibit IL-8 release from TNF-α-stimulated HUVEC compared with FH-derived HDL₁ with a lower triglyceride content. Previous observations from studies with subjects with FH (25) showed that a TG-enriched LDL particle is more atherogenic because it increases the release of proinflammatory cytokines. Taken together, we suggest that an increase in the triglyceride content of the HDL₃ generates a more proatherogenic lipoprotein particle with less ability to inhibit expression and release of proinflammatory cytokines and to delay the atherosclerotic process.

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Finally, we demonstrated that FH-derived HDL₃ with a high triglyceride content have a reduced capacity to induce cholesterol efflux from lipoprotein macrophages, comparing FH-derived HDL₃ with less triglyceride content. What is even more interesting is that the degree of inhibition of IL-8 release from HUVEC was correlated with the ability of HDL₃ to promote cholesterol efflux. Other studies showed that a TG-rich HDL has an attenuated capacity to accept cholesterol (26,27) and that a proinflammatory HDL is associated with reduced ability to promote cholesterol efflux (23). Abnormal composition and alternations in the HDL₂ were documented in subjects with type 2 diabetes (27). However, to our knowledge, the finding that a TG-rich HDL₃ derived from FH-patients has an altered cholesterol efflux capacity and proinflammatory properties was not described previously. In vivo, such a TG-rich HDL₃ might enhance the accumulation of lipid-rich macrophages in the artery wall while simultaneously increasing the release of IL-8 from both the endothelium and the monocytes/macrophages. Consequently, this could enhance the recruitment of inflammatory cells, causing plaque progression and subsequently plaque instability. However, the mechanism by which TG-rich HDL₃ reduces the capacity to accept cholesterol is not yet known, although an altered conformation of apolipoprotein A-I was suggested (28).

In conclusion, our study demonstrates that compared with healthy controls, FH-patients have greater concentrations of low-grade proinflammatory mediators, which are associated with total and LDL cholesterol concentrations. In addition, FH-patients have an unfavorable antioxidant profile. Furthermore, this study adds new insight into the atheroprotective function of HDL₃ in FH-patients by demonstrating that TG-rich HDL₃ particles have proatherogenic properties compared with HDL₃ containing fewer triglycerides. These findings might explain in part the phenotypic variation in FH-patients with an identical LDL-receptor mutation. The reduced anti-inflammatory capacity and the decreased ability to promote cholesterol efflux in TG-rich HDL₃ may contribute to progression of atherosclerosis in vivo. This illustrates the importance of triglyceride-lowering treatment in FH-patients with elevated serum triglycerides so that the atheroprotective function of the HDL₃ particle is preserved.

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LITERATURE CITED
27. Gowri MS, Van der Westhuysen DR, Bridges SR, Anderson JW. Decreased protection by HDL from poorly controlled type 2 diabetic subjects against LDL oxidation may be due to the abnormal composition of HDL. Arterioscler Thromb Vasc Biol. 1999;19:2226–33.