Preβ₁-high-density lipoprotein increases in coronary artery disease

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Preβ₁-HDL promotes cholesterol efflux from cell membranes. Its plasma concentration is regulated by lecithin: cholesterol acyltransferase (LCAT). To clarify whether the concentration of preβ₁-HDL changes in coronary artery disease (CAD), we determined the distribution of apolipoprotein A-I (apoA-I) among HDL subfractions in 20 CAD patients and 20 healthy controls, using nondenaturing two-dimensional gel electrophoresis. We found that CAD patients had significantly higher concentrations of preβ₁-HDL than the controls [7.6% ± 3.4% vs 4.6% ± 2.3% of apoA-I (P < 0.01)]. Even after correcting for apoA-I concentrations, this increase remained significant [87 ± 37 vs 63 ± 28 mg/L apoA-I (P < 0.05)]. The mean LCAT activity concentration was significantly lower (P < 0.05) in CAD patients than in controls. These findings, that preβ₁-HDL concentrations increase in CAD, strongly suggest that the process of reverse cholesterol transport could be altered in CAD.

**INDEXING TERMS:** reverse cholesterol transport • lecithin: cholesterol acyltransferase • lipoproteins • apolipoproteins • atherosclerosis • ischemic heart disease

High-density lipoprotein (HDL) is well-known as the negative risk factor for coronary artery disease (CAD).⁴ Several epidemiological studies have shown that plasma HDL-cholesterol concentrations are inversely correlated with the incidence of CAD [1-3]. This antiatherogenic effect of HDL is related to the transport of excess peripheral cholesterol to the liver [4].

Although HDL is defined as lipoproteins with the density range of 1.063-1.210 kg/L [5], HDL particles are heterogeneous in size, apolipoprotein composition, and function [6-8]. Preβ₁-HDL, a putative discoid-shaped HDL that migrates with preβ mobility in agarose gel electrophoresis [8], contains apolipoprotein A-I (apoA-I) but no apoA-II. The smallest preβ-HDL, preβ₁-HDL picks up free cholesterol efficiently from cell membranes [9]. This ability of preβ₁-HDL is lost when cell membranes are treated with protease, thus suggesting receptor-mediated cholesterol uptake through preβ₁-HDL [10]. Plasma preβ₁-HDL decreases during incubation with lecithin:cholesterol acyltransferase (LCAT) activity [11-13], but this decrease can be blocked when the plasma is incubated with fibroblasts, smooth muscle cells, or macrophages (but not with erythrocytes) [13]. Preβ₁-HDL maintains its plasma concentration when incubated with these cells probably because cell membranes supply free cholesterol to preβ₁-HDL. Considering these features of preβ₁-HDL, we thought it likely that preβ₁-HDL concentrations change in atherosclerotic diseases such as CAD.

To examine this possibility, we determined the distribution of apoA-I among HDL subfractions by using nondenaturing two-dimensional gel electrophoresis to assay plasma from 20 CAD patients and compared the results with the subfractions found in 20 normolipidemic control subjects.

**Materials and Methods**

**SUBJECTS AND BLOOD SAMPLES**

We screened 23 consecutive CAD patients whose angiograms gave evidence of coronary atherosclerosis, with at least one lesion occluding 75% of the coronary artery diameter. Three patients were excluded because they took cholesterol-lowering drugs. Thus, 20 CAD patients (11 men, 9 women; ages 49-82 years) participated in this study. We then chose at random 20 sex-matched normolipidemic subjects who had no history of atherosclerotic diseases (ages 37-80 years). Each subject gave informed consent at enrollment in the study, and this protocol was approved by our institutional review board.

After an overnight fast, 2 mL of blood was collected from each subject into an ice-cooled tube containing K₂-EDTA (Sigma, St. Louis, MO). K₂-EDTA did not affect apoA-I distribution among HDL subfractions (although in previous reports [14] we used streptokinase or sodium citrate as antico-

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¹Nonstandard abbreviations: CAD, coronary artery disease; LCAT, lecithin: cholesterol acyltransferase; and apo, apolipoprotein.

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agulant). Plasma was obtained after a 30-min centrifugation at 0 °C and 2000g. To prevent interconversions of HDL, we stored the samples on ice-water until the electrophoresis procedure. All samples were run within 24 h on two-dimensional gels as described below. We confirmed that HDL subfractions did not change significantly under these conditions (data not shown).

METHODS

Lipid and apolipoprotein concentrations and LCAT activity. Concentrations of total cholesterol and triglyceride were measured enzymatically with commercially available kits (Determiner-L-TCN, Kyowa Medex, Tokyo, Japan; and L-type Wako TG-H, Wako Pure Chemical Co., Osaka, Japan, respectively). HDL-cholesterol was determined in whole plasma by an enzymatic technique (HDL-C: 2-Daiichi; Daiichi Chemical Co., Tokyo, Japan), after precipitation of apoB-containing lipoproteins with phosphotungstic acid/dextran sulfate. Plasma concentrations of apoA-I and apoA-II were measured by a turbidimetric immunnoassay (Apo Auto AI, All; Daiichi Chemical Co.) according to the manufacturer’s instructions. We determined LCAT activity by the endogenous substrate method with a commercial kit (Nihon-shoiji, Osaka, Japan).

Nondenaturing two-dimensional gel electrophoresis. Nondenaturing two-dimensional fractionation of HDL particles was carried out as previously published with slight modifications [15]. In the first dimension, each sample (20 μL) was separated by charge on a 7.5 g/L agarose strip (2.5 cm × 12.5 cm × 1.1 mm thick) in 50 mmol/L barbital buffer (pH 8.6) at 200 V. In the second dimension, HDL was further fractionated by particle size on 2–15% gradient polyacrylamide gel in Tris–glycine buffer (pH 8.3), without sodium dodecyl sulfate, for 2000 V·h. Fractionated HDL was electroblotted to the nitrocellulose membranes (0.45-μm pore size; Sartorius, Göttingen, Germany) in Tris–glycine–methanol buffer (pH 8.3) at 30 V, overnight. Throughout the experiments, the temperature was maintained at 0 °C to avoid interconversion of HDL subfractions.

Quantification of HDL subfractions. HDL subfractions were localized by Western blotting with 125I-labeled anti-human apoA-I antibodies. Each spot was excised from the nitrocellulose membrane with a razor blade. The radioactivity was counted by γ-spectrometry [16]. The concentration of each HDL subfraction was expressed as the percentage of total apoA-I (%A-I; relative concentration). We also calculated the absolute concentration for each fraction by multiplying its percentage by the plasma concentrations of apoA-I (mg/L apoA-I).

Statistical analysis. Data are given as mean ± SD. Statistical comparisons were made with Student’s t-test unless otherwise stated. P <0.05 was considered significant.

Results

Lipid and apolipoprotein concentrations and LCAT activity. Concentrations of total cholesterol and triglycerides were similar between the control and CAD groups. However, concentrations of HDL-cholesterol, apoA-I, and apoA-II were significantly lower in the CAD patients than in the control group (Table 1). LCAT activity was also significantly lower in the CAD patients than in the controls.

Nondenaturing two-dimensional gel electrophoresis. Using two-dimensional gel electrophoresis followed by apoA-I immunoblotting, we classified HDL into six subfractions. The preβ-HDL was separated into three subgroups (preβ1, preβ2, and preβ3), as
was α-HDL (HDL$_{2b}$, HDL$_{2a}$, and HDL$_1$). LDL was also visible as a small spot in this system (Fig. 1).

**Concentrations of HDL subfractions.** In the typical patients with CAD, preβ$_1$-HDL spots were slightly bigger than those in the controls (Fig. 1). Moreover, the preβ$_1$-HDL concentration was 1.5 times higher in the CAD group than in the control group (Fig. 2, top). No other subfraction differed significantly between the two groups. After we corrected for the plasma apoA-I concentrations, the preβ$_1$-HDL concentrations were still significantly higher in the CAD patients than in the controls (Fig. 2, bottom). On the other hand, concentrations of the α-HDL subgroups (HDL$_{2b}$, HDL$_{2a}$, and HDL$_1$) were significantly lower in the CAD subjects than in the controls.

**Relation between LCAT activity and preβ$_1$-HDL concentrations.** To elucidate the effect of LCAT activity on preβ$_1$-HDL concentrations, we divided the CAD patients into two subgroups, according to their LCAT activity. CAD patients with low LCAT activity had 25% higher preβ$_1$-HDL concentrations than those with high LCAT activity—although this difference was not significant (Fig. 3). However, the preβ$_1$-HDL concentrations in CAD patients with low LCAT activity were still significantly higher than those in the control group.

**Discussion**

We have shown, using nondenaturing two-dimensional gel electrophoresis, that the concentrations of preβ$_1$-HDL increase in CAD. Not only were preβ$_1$-HDL concentrations (%A-I) significantly higher in the CAD patients than in the controls (Fig. 2, top), but also this increase remained significant after correcting for plasma apoA-I concentrations (Fig. 2, bottom)—even though the apoA-I concentrations were lower in the former than in the latter (Table 1).

Previous reports showed that total preβ-HDL increased in atherogenic conditions. Ishida et al. [16] found that total preβ-HDL made up as much as 63% of plasma apoA-I in lipoprotein disorders such as familial hypercholesterolemia, lipoprotein lipase deficiency, apoC-II deficiency, LCAT deficiency, and familial combined hyperlipidemia. In addition, total preβ-HDL increased within 7 days in mice fed a high-fat diet (containing 12.5 g/kg cholesterol) and then decreased to a stable concentration higher than the baseline value [11]. In their study [11, 16], preβ$_1$-HDL concentrations probably increased because ~50% of total preβ-HDL is preβ$_1$-HDL in normolipidemic subjects.

We speculate that apoA-I may redistribute more in preβ$_1$-HDL than in any other HDL subfraction during atherogenic conditions. Two possible mechanisms could account for such a redistribution of apoA-I. The first possibility, impaired consumption of preβ$_1$-HDL by LCAT, was evident in the present study, the LCAT activity being significantly lower in CAD...
patients than in controls (Table 1). Moreover, only CAD patients with low LCAT activity showed significantly higher preβ1-HDL concentrations than the controls (Fig. 3). The close relationship between preβ-HDL concentration and LCAT activity was also seen in both in vivo and in vitro experiments. In normolipidemic healthy subjects, total preβ-HDL concentrations were significantly inversely correlated with LCAT activity [12], and in one patient with LCAT deficiency, total preβ-HDL concentrations markedly increased [16], supporting this hypothesis. In previous in vitro experiments, we showed that preβ1-HDL markedly decreased after a 90-min incubation at 37 °C [13]; however, this decrease was completely blocked by either LCAT inhibitor 5,5′-dithio-bis(2-nitrobenzoic acid) or specific antibodies against LCAT. Other investigators have also observed the same behavior of total preβ-HDL concentration [11, 12]. Low LCAT activity, therefore, may be responsible for the high preβ1-HDL concentrations in CAD patients. It is reasonable that preβ1-HDL will accumulate in plasma if LCAT does not efficiently esterify the free cholesterol of preβ1-HDL.

The second possible mechanism is an enhanced production or recycling of preβ1-HDL in CAD. The “free apoA-I” can be dissociated from α-HDL spontaneously or by lipase activity. In fact, some investigators have observed free apoA-I or lipid-poor apoA-I complexing during the lipolytic process [17]. In vitro experiments have shown that free apoA-I picks up free cholesterol from the macrophages to form lipid-apoA-I complexes with preβ-mobility in agarose gel electrophoresis [18]. These particles were similar to preβ1-HDL in their function [18]. Moreover, in the presence of atheromatous plaques, which must be rich in cholesterol-loaded macrophages, the free apoA-I is likely to interact and form preβ1-HDL in situ. When cell membranes of macrophages were present during the 37 °C-incubation with LCAT, the concentration of preβ1-HDL did not decrease [13]. Such a preventive effect strongly suggests that preβ1-HDL is recycled in the presence of cell membranes of macrophages. Therefore, we speculate that macrophages may promote in-situ production or recycling of preβ1-HDL. In the present study, some patients had high concentrations of preβ1-HDL despite high LCAT activity. In such cases, the high concentration of preβ1-HDL probably resulted from its overproduction in the atherosclerotic plaques.

Although preβ1-HDL concentrations clearly increase in CAD, the clinical significance of the preβ1-HDL value is not clear. Perhaps apoA-I redistributes more in preβ1-HDL than in any other HDL fraction to compensate for the accumulation of cholesterol in atherosclerotic plaques. Further study is required to clarify the exact mechanism regulating preβ1-HDL concentrations. In conclusion, we have shown that concentrations of preβ1-HDL increase in CAD patients. These findings strongly suggest that the reverse cholesterol transport could be altered in CAD.

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References