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High Pre-β_1 HDL Concentrations and Low Lecithin: Cholesterol Acyltransferase Activities Are Strong Positive Risk Markers for Ischemic Heart Disease and Independent of HDL-Cholesterol

Amar A. Sethi,[1*] Maureen Sampson,[2] Russell Warnick,[3] Nehemias Muniz,[4] Boris Vaisman,[1] Børge G. Nordestgaard,[5,6] Anne Tybjærg-Hansen,[6,7] and Alan T. Remaley[1]

BACKGROUND: We hypothesized that patients with high HDL-cholesterol (HDL-C) and ischemic heart disease (IHD) may have dysfunctional HDL or unrecognized nonconventional risk factors.

METHODS: Individuals with IHD (Copenhagen University Hospital) and either high HDL-C (n = 53; women ≥735 mg/L; men ≥619 mg/L) or low HDL-C (n = 42; women ≤387 mg/L; men ≤341 mg/L) were compared with individuals without IHD (Copenhagen City Heart Study) matched by age, sex, and HDL-C concentrations (n = 110). All participants had concentrations within reference intervals for LDL-C (<1600 mg/L) and triglyceride (<1500 mg/L), and none were treated with lipid-lowering medications. Pre-β_1 HDL and phospholipid transfer protein concentrations were measured by using commercial kits and lecithin:cholesterol acyltransferase (LCAT) activity by using a proteoliposome cholesterol esterification assay.

RESULTS: Pre-β_1 HDL concentrations were 2-fold higher in individuals with IHD vs no IHD in both the high [63 (5.7) vs 35 (2.3) mg/L; $P < 0.0001$] and low HDL-C [49 (5.0) vs 27 (1.5) mg/L; $P = 0.001$] groups. Low LCAT activity was also associated with IHD in the high [95.2 (6.7) vs 123.0 (5.3) $\mu mol \cdot L^{-1} \cdot h^{-1}$; $P = 0.002$] and low [93.4 (8.3) vs 113.5 (4.9) $\mu mol \cdot L^{-1} \cdot h^{-1}$; $P = 0.03$] HDL-C groups. ROC curves for pre-β_1 HDL in the high–HDL-C groups yielded an area under the curve of 0.71 (95% CI: 0.61–0.81) for predicting IHD, which increased to 0.92 (0.87–0.97) when LCAT was included. Similar results were obtained for low HDL-C groups. An inverse correlation between LCAT activity and pre-β_1 HDL was observed ($r^2 = 0.30$; $P < 0.0001$) in IHD participants, which was stronger in the low HDL-C group ($r^2 = 0.56$; $P < 0.0001$).

CONCLUSIONS: IHD was associated with high pre-β_1 HDL concentrations and low LCAT levels, yielding correct classification in more than 90% of the IHD cases for which both were measured, thus making pre-β_1 HDL concentration and LCAT activity level potentially useful diagnostic markers for cardiovascular disease.

LDL and HDL are well-established independent risk factors for cardiovascular disease *(1)*. Several clinical trials have shown that lipid-lowering drugs aimed at LDL cholesterol (LDL-C)[8] reduce cardiovascular events by 30%–45% *(2, 3)*. The large residual risk in treated individuals may be partially explained by low HDL-C *(4)*, but recent reports have suggested that increased HDL-C does not always protect against cardiovascular disease *(5)* and can sometimes be associated with increased coronary events *(6)*.

Although epidemiologic studies have shown that low HDL-C is a negative risk factor, raising HDL-C pharmacologically has not been definitively established to protect against ischemic heart disease (IHD) *(5, 7)*. This was especially evident from the recent study of the cholesteryl ester transfer protein (CETP)-inhibitor torcetrapib, which increased HDL-C concentrations but did not reduce cardiovascular events *(7)*. A possible explanation for these contradictory findings may be

[1] NIH, National Heart Lung and Blood Institute, Lipoprotein Metabolism Section and [2] Clinical Center, Department of Laboratory Medicine, Bethesda, MD; [3] Berkeley HeartLab, Burlingame, CA; [4] Quantimetrix, Redondo Beach, CA; [5] Herlev Hospital, [6] Copenhagen City Heart Study, and [7] Rigshospitalet, Copenhagen University Hospital, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.
* Address correspondence to this author at: Pacific Biomarkers, 220 West Harrison St., Seattle, WA 98119. Fax 206-298-9838; e-mail amars@pacbio.com.

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[8] Nonstandard abbreviations: LDL-C, LDL cholesterol; IHD, ischemic heart disease; CETP, cholesteryl ester transfer protein; ABCA1, ATP binding-cassette transporter 1; apoB, apolipoprotein B; S-GGE, segmented gradient-gel electrophoresis; AUC, area under the curve; PLTP, phospholipid transfer protein; LCAT, lecithin:cholesterol acyltransferase; 2D, 2 dimensional.

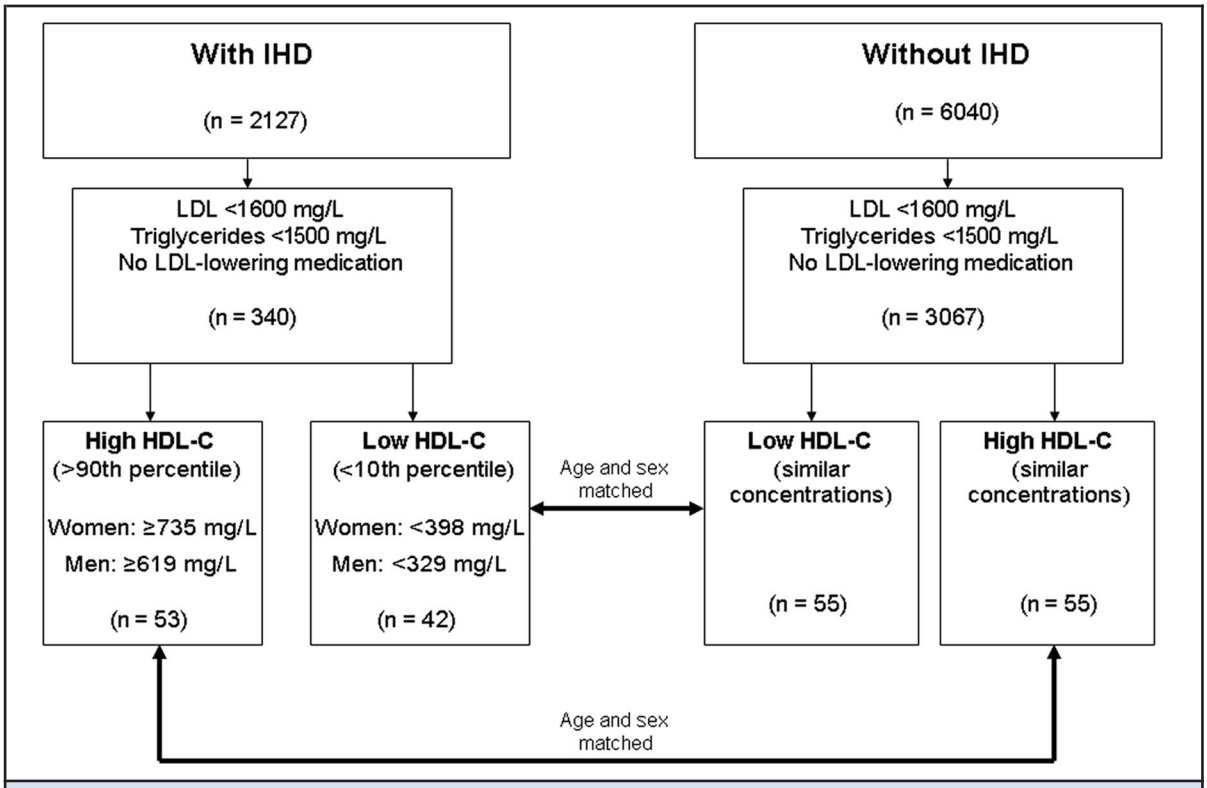

Fig. 1. Patient selection criteria.

Individuals with and without IHD were selected on the basis of concentrations that were below to within the reference interval for LDL-C and triglycerides. HDL-C ≥735 mg/L for women and ≥619 mg/L for men were the 90th percentile cutoffs for 2127 patients with IHD. HDL-C <398 mg/L for women and <329 mg/L for men were the 10th percentile cutoffs for 3067 individuals without IHD. Cases and controls were age and sex matched and no study participants were on lipid-lowering medications.

that HDL becomes "dysfunctional" and may lose some of its antiatherogenic properties *(8–10)*. For example, HDL was recently reported to become impaired in the ATP binding cassette transporter 1 (ABCA1)-dependent cholesterol efflux when oxidatively damaged by myeloperoxidase *(11)*. Furthermore, HDL may sometimes acquire proinflammatory properties and can perhaps even actively contribute to the pathogenesis of atherosclerosis *(12)*. Overall, these study findings suggest that the classification of HDL as either anti- or proatherogenic may require a more complete analysis of the components of HDL besides its cholesterol content and/or an analysis of the pathways by which HDL mediates its antiatherogenic effects.

One of the main goals of this study was to examine other biomarkers of HDL besides HDL-C that could be measured with automated and readily implemented methods in a routine clinical laboratory for cardiovascular risk assessment. We used an age- and sex-matched 2 × 2 design in patients with high and low HDL-C with and without IHD to examine their HDL subfraction distribution, HDL lipid composition, and the major apolipoproteins and enzymes associated with HDL, with the expectation that some of these patients may have dysfunctional HDL, perhaps as a consequence of a change in their protein or lipid composition. Two biomarkers related to HDL, pre-β_1 HDL concentration and LCAT activity, were found to be associated with IHD in individuals with both low and high HDL-C. These results also provide new insights into the antiatherogenic mechanisms of HDL.

Methods

STUDY PARTICIPANT SELECTION

Individuals with IHD were identified from 2127 patients who were initially recruited from 1991 through 2004 from the greater Copenhagen area in Denmark (Fig. 1). More than 99% of the participants were whites of Danish descent. All were referred to Copenhagen University Hospital for coronary angiography, because of clinical suspicion of IHD. The diagnosis of IHD was

determined by experienced cardiologists based on a positive history of angina pectoris plus at least 1 of the following criteria: stenosis/atherosclerosis on coronary angiography, a previous myocardial infarction, or significant myocardial ischemia on a bicycle exercise electrocardiography test. Individuals without IHD were identified from the Copenhagen City Heart Study, which is a prospective cardiovascular study of individuals randomly selected on the basis of the Danish Central Population Register Code to reflect the adult general population of Denmark *(13)*. Blood samples collected in the fourth examination from 6040 individuals considered to be without IHD based on clinical history and the national Danish Patient Registry and the national Danish Causes of Death Registry were used in this study (Fig. 1). All participants gave informed consent and were collected under study protocols approved by Danish ethics committees and by Herlev Hospital, Copenhagen University Hospital.

EXPERIMENTAL DESIGN

A flow chart of the experimental 2 × 2 design is shown in Fig. 1. All selected individuals had concentrations below to within the reference interval for LDL-C (<1600 mg/L) and triglycerides (<1500 mg/L), based on ATP III guidelines *(14)*, and were receiving no drug treatment for lipids. Patients with IHD and high HDL-C concentrations (>90th percentile) were selected (n = 53; women, ≥735 mg/L; men, ≥619 mg/L) and matched by age and sex to a control group without IHD, with same HDL-C cutoff concentrations (n = 55). Likewise, individuals without IHD and low HDL-C concentrations (<10th percentile) were selected (n = 55; women, ≤398 mg/L; men, ≤329 mg/L) and matched by age and sex to a group with IHD and similar low HDL-C concentrations (n = 42) (Fig. 1).

LABORATORY ANALYSIS

Analysis of HDL apolipoproteins and lipids was done on serum stored at −80 °C after apolipoprotein-B (apoB)-containing lipoproteins were removed by precipitation using dextran-sulfate on beads (Polymedco), followed by analysis of the supernatant on Cobas-Fara (Roche) for apoA-I, apoA-II, apoE, triglycerides, total cholesterol, free cholesterol, and phospholipids (Wako Chemicals). Total cholesterol, LDL, HDL, and triglycerides were measured as described elsewhere *(15)*. HDL subclasses were examined in plasma by use of Lipoprint® (Quantimetrix), with linear gel electrophoresis, and by segmented gradient-gel electrophoresis (S-GGE) (Berkeley HeartLab). The S-GGE method is based on the electrophoretic separation of lipoproteins, which uses progressively tighter acrylamide matrices to create decreasing gel porosity. The Lipoprint HDL system uses a discontinuous, single-concentration polyacrylamide gel electrophoresis method that separates the various HDL subfractions based on size into large, intermediate, and small. The system includes precasted tube gels and proprietary data analysis software to determine the HDL subfraction concentrations based on area under the curve (AUC). Pre-β_1 HDL, phospholipid transfer protein (PLTP), and CETP concentrations were measured by using commercial kits from Polymedco, Biovision, and Roar Biomedical, respectively. Lecithin:cholesterol acyltransferase (LCAT) activity was measured with proteoliposomes made with the apoA-I mimetic peptide ETC-642 *(16)*. The mean CVs for LCAT, PLTP, and CETP were 5.9%, 18.9%, and 12.6%, respectively, for the intraassay variation and 11.2%, 8.7%, and 7.9%, respectively, for the interassay variation. Results shown are the mean of at least duplicate analysis.

STATISTICAL ANALYSES

ANOVA and *t*-tests were used for comparison between means, and analysis of covariance was used to adjust continuous variables for other covariables. Fisher exact tests were used to examine differences in frequencies. ROC curves were performed to assess diagnostic accuracy *(17)*. A 2-sided *P*-value <0.05 was considered significant, and Sidak adjustment was used to correct for multiple comparisons.

Results

Characteristics of participants are shown in Table 1. Overall, men were more frequent in our study population, representing about 70% in each group. Lipid and lipoprotein concentrations and other cardiovascular risk markers did not statistically differ between individuals with and without IHD for both the high and low HDL-C groups. The combined low HDL-C groups, however, were found to have higher triglyceride concentrations ($P = 0.0001$) and body mass index ($P = 0.0001$) compared to the combined high HDL-C groups.

IHD participants with high HDL-C had lower free-cholesterol percentages by weight of the HDL particle compared to their control group without IHD [2.9 (0.84)% vs 3.3 (0.79)%; $P = 0.02$] (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol56/issue7). Likewise, triglyceride percentages by weight of the HDL particle were lower in IHD participants with low HDL-C compared to their respective control group [2.1 (0.81)% vs 2.6 (1.1)%; $P = 0.02$] (see online Supplemental Table 1). ApoE enrichment per HDL particle was observed in IHD patients with low HDL-C ($P = 0.05$) compared to the control group (see online Supplemental Table 1). In the combined

Table 1. Characteristics of study participants.[a]

	High HDL-C[b]			Low HDL-C[c]		
	With IHD (n = 53)	No IHD (n = 55)	P[d]	With IHD (n = 42)	No IHD (n = 55)	P[d]
Age, y	63.1 (10.3)	62.6 (10.3)	1.00	61.5 (9.3)	62.4 (9.7)	1.00
Women, %	30.2	29.1	1.00	31.0	29.1	1.00
Total cholesterol, mg/L	2081 (25.7)	2073 (31.2)	1.00	1823 (29.4)	1669 (30.9)	0.13
HDL-C, mg/L	784 (14.3)	805 (14.1)	0.99	324 (5.2)	337 (5.8)	0.85
LDL-C, mg/L	1134 (26.3)	1188 (28.0)	0.93	1209 (30.6)	1179 (25.4)	1.00
Triglycerides, mg/L	821 (30.0)	741 (25.2)	0.65	1049 (31.1)	1077 (29.1)	1.00
Body mass index, kg/m^2	24.8 (4.2)	23.6 (3.1)	0.48	26.0 (3.5)	27.9 (5.1)	0.35
Smokers, %	27.1	42.6	0.15	26.8	34.5	0.51
Diabetes mellitus, %	7.7	5.5	0.71	14.3	7.3	0.32
Treated for hypertension, %	22.6	12.7	0.21	31.0	29.1	1.00
IHD treatment plan						
PTCA[e] or CABG, %	58			69		
Medical treatment, %	42			31		

[a] Values are mean (SD) or %. All selected participants had concentrations within reference intervals for LDL-C (<1600 mg/L) and triglycerides (<1500 mg/L) and no ongoing or previous treatment with lipid-lowering medications.
[b] 90th percentile based on 2127 patients with IHD (women, ≥735 mg/L; men, ≥619 mg/L).
[c] Lowest 10th percentile based on 6040 individuals without IHD (women, ≤398 mg/L; men, ≤329 mg/L).
[d] *P* values are for *t*-test between means corrected for multiple comparisons and Fisher exact test between frequencies.
[e] PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass graft.

low HDL-C groups, enrichment per HDL particle was observed for all of the apolipoproteins ($P < 0.0001$ for all) and triglycerides ($P < 0.0001$), whereas individuals in these groups had less free cholesterol ($P < 0.0001$), cholesterol ester ($P < 0.0001$), and phospholipids ($P < 0.0001$) when compared to the combined high HDL-C groups (see online Supplemental Table 1). The latter data suggest the presence of higher numbers of small, more dense HDL particles in the low HDL-C groups compared to the high HDL-C groups. The distribution of HDL subfractions in the 4 groups was examined by use of 2 different techniques, S-GGE and the Lipoprint system. Results of both methods showed that the overall concentration of HDL was increased for all subfractions, particularly for the largest size HDL subclasses (HDL_{2b}), when all individuals with high HDL-C were compared to the combined low HDL-C groups (S-GGE: HDL_{2b}, $P = 0.001$; HDL_{2a}, $P = 0.001$; HDL_{3a}, $P = 0.001$; HDL_{3b}, $P = 0.001$; HDL_{3c}, $P = 0.01$; Lipoprint: large, $P = 0.001$; intermediate, $P = 0.001$; small, $P = 0.001$). In contrast, no significant differences were observed in S-GGE results when individuals with IHD were compared to their control group without IHD for both the high (HDL_{2b}, $P = 0.37$; HDL_{2a}, $P = 0.85$; HDL_{3a}, $P = 0.23$; HDL_{3b}, $P = 0.96$; HDL_{3c}, $P = 0.68$) and low HDL-C groups (HDL_{2b}, $P = 0.45$; HDL_{2a}, $P = 0.59$; HDL_{3a}, $P = 0.81$; HDL_{3b}, $P = 0.82$; HDL_{3c}, $P = 0.22$). We observed a similar lack of difference in HDL subfractions by use of the Lipoprint method when we compared the 2 IHD disease groups to their control groups (data not shown).

Pre-β_1 HDL was measured by an ELISA *(18)*, because neither S-GGE nor the Lipoprint method can detect pre-β_1 HDL. Study participants with IHD and high HDL-C concentrations had an almost 2-fold [63 (5.7) vs 35 (2.3) mg/L] increase in pre-β_1 HDL compared with participants without IHD ($P < 0.0001$; Fig. 2A). Similarly, patients with low HDL-C concentrations and IHD also had an almost 2-fold [49 (5.0) vs 27 (1.5) mg/L] increase in pre-β_1 HDL compared with individuals without IHD ($P < 0.0001$; Fig. 2B). A poor correlation was observed between pre-β_1 HDL and HDL-C ($r^2 = 0.04$) when all groups were combined.

Next, we examined several HDL-associated enzymes that can affect pre-β_1 HDL concentrations (Fig. 3). In individuals with IHD compared to their control groups, LCAT activity was reduced by approximately 23% [95.2 (6.7) vs 123.0 (5.3) $\mu mol \cdot L^{-1} \cdot h^{-1}$; $P = 0.002$] in the high HDL-C group and by 18% [93.4 (8.3) vs 113.5 (4.9) $\mu mol \cdot L^{-1} \cdot h^{-1}$; $P = 0.03$] in the low HDL-C group (Fig. 3, top panel). Furthermore, pre-β_1 HDL concentrations differed between individ-

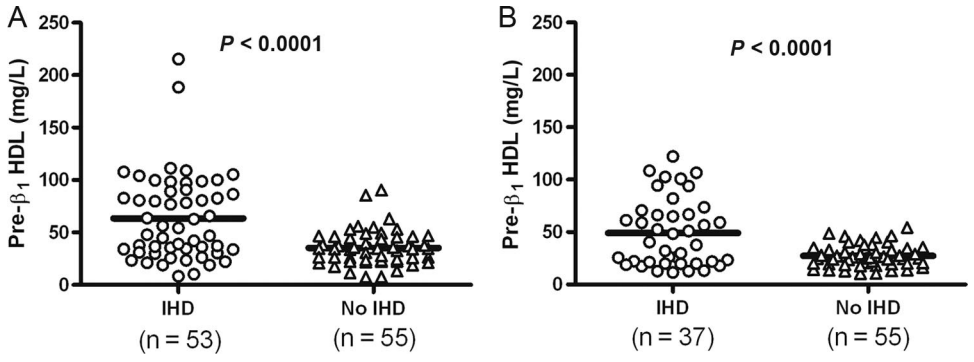

Fig. 2. Association of pre-β_1 HDL concentrations with IHD.

Pre-β_1 HDL serum concentrations are shown as a function of IHD in individuals with high (A) or low HDL-C (B). (○) with IHD; (△) without IHD.

uals with and without IHD when we examined them as a function of LCAT activity (Fig. 4). Individuals with IHD in the lowest LCAT tertile had higher pre-β_1 HDL than individuals without IHD ($P < 0.0001$, Fig. 4A, far left). It should be noted, however, that increased pre-β_1 HDL was observed across all LCAT tertiles in individuals with IHD, a result that may not have reached statistical significance because of the relatively small sample size. Similar results were observed for the low HDL-C groups (Fig. 4B), with the IHD participants in the lowest LCAT tertile having significantly higher pre-β_1 HDL concentrations than their control group, which suggests that the pre-β_1 HDL increment in patients with IHD is independent of HDL-C concentrations but is associated with low LCAT activities. No statistically significant differences in pre-β_1 HDL concentrations were found for any LCAT tertile when the high and low HDL-C groups were compared (data not shown). Individuals in the high HDL-C group with IHD also had an approximately 26% [19.8 (0.7) vs 15.7 (0.6) $\mu mol \cdot L^{-1} \cdot h^{-1}$; $P < 0.0001$] increase in PLTP activity compared to their control group (Fig. 3, middle panel). Interestingly, we observed no differences for CETP activity in any of the 4 groups, even when we compared the high with the low HDL-C groups (Fig. 3, bottom panel).

ROC curves were generated for all of the laboratory markers examined in this study to determine the possible diagnostic utility of the markers for distinguishing the 2 IHD groups from their control non-IHD groups, but only pre-β_1 HDL concentration and LCAT activity showed modest utility in this regard. ROC curves for pre-β_1 HDL had an AUC of 0.71 (95% CI, 0.61–0.81) for the high HDL-C groups and 0.67 (0.55–0.79) for the low HDL-C groups (Fig. 5, A and B), which is comparable to what has been reported in a more general population for conventional cardiovascular risk markers as HDL-C and LDL-C *(17)*. Similar results were obtained when we examined ROC curves for LCAT activity, with AUC of 0.67 (0.57–0.77) and 0.62 (0.50–0.74) for the high and low HDL-C group comparisons, respectively (data not shown). After we adjusted pre-β_1 HDL for LCAT activity, we observed a marked improvement in correctly classified individuals with and without IHD. For the high HDL-C group, the AUC was 0.92 (0.87–0.97) for distinguishing between the disease and nondisease group, whereas a similar AUC of 0.91 (0.85–0.97) was obtained for distinguishing between the disease and nondisease group for the low HDL-C groups (Fig. 5, C and D, respectively). At a sensitivity of 90%, a combination of these 2 tests would yield specificities of 75% and 60%, for the high and low HDL-C groups, respectively, for correctly classifying individuals with IHD (Fig. 5, C and D).

Discussion

In study participants who developed IHD despite having LDL-C and high HDL-C concentrations that were low to within reference intervals, we expected that examination would reveal that some of these patients had dysfunctional HDL. We also examined a group of patients with IHD and low HDL-C, with the expectation that they may have HDL with relatively normal function but at insufficient concentrations to protect against IHD. Our results showed an association between high pre-β_1 HDL concentration and low LCAT activity in patients with IHD. This association was observed for both the high and low HDL-C groups, which suggests that the findings are independent of the HDL-C concentrations. These results indicate that the development of IHD may be more closely related to a

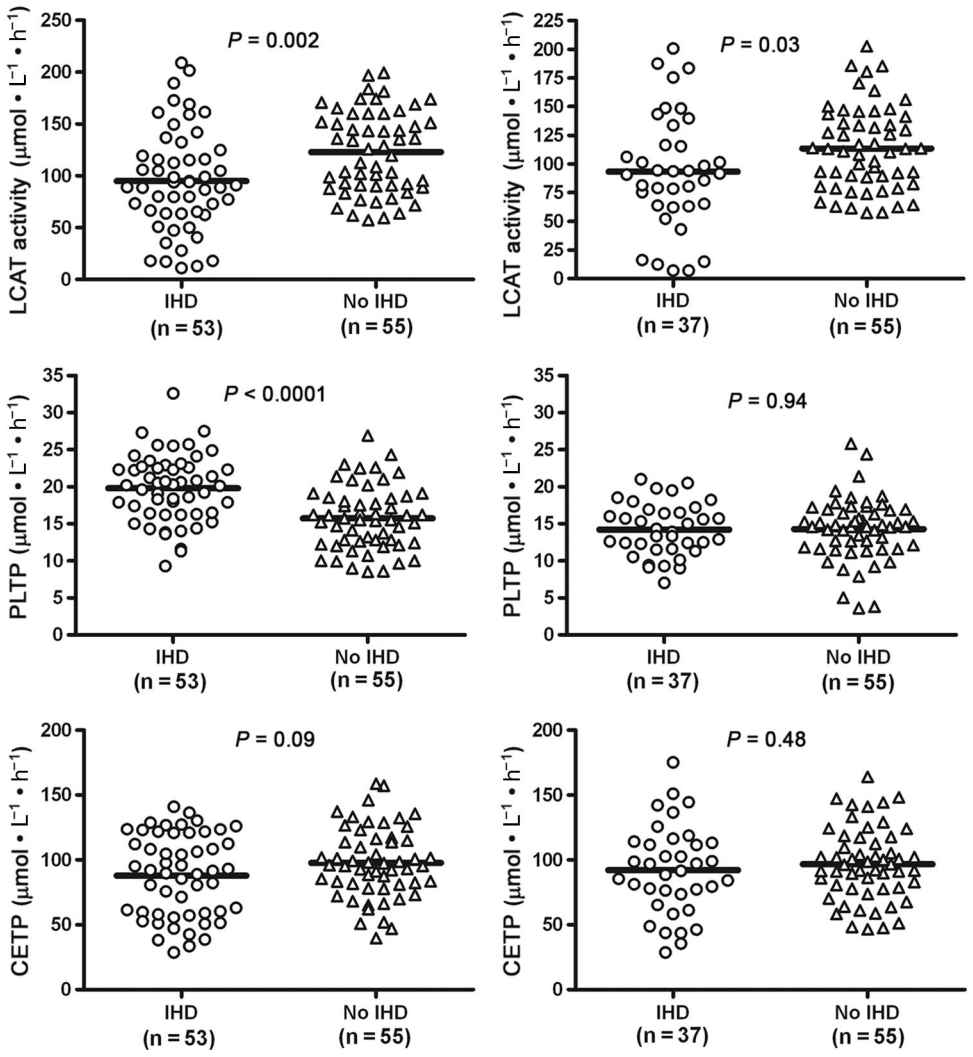

Fig. 3. Association of HDL-associated enzymes with IHD.

Serum LCAT activity (top panels), PLTP activity (middle panels), and CETP activity (bottom panels) are shown as a function of IHD in individuals with high (left panels) or low HDL-C concentrations (right panels). (○) with IHD; (△) without IHD.

combination of multiple factors in the reverse cholesterol transport pathway rather than one particular metric, such as HDL-C.

Although minor differences were observed in the protein and lipid composition components of HDL, these differences were relatively small, and none were useful as diagnostic discriminators of IHD. Enrichment of apoE on HDL, which was only marginally significant in our study, has previously been associated with IHD *(19)*. ApoE has been proposed as a marker for dysfunctional HDL *(20)*, although it is usually also associated with increased triglycerides *(21)*, which was not the case in the present study (see online Supplemental Table 1). In contrast, we found that individuals with IHD and low HDL-C had lower triglyceride concentrations per HDL particle compared to their control group, which suggests smaller HDL particles in the disease group.

Many previous studies have shown that large lipid-rich species of HDL, such as HDL_{2b}, are negative risk factors for IHD, and in several studies HDL_{2b} was shown to be superior to HDL-C for prediction of IHD risk *(22, 23)*. It was recently also suggested that small HDL particle size is associated with several features of the metabolic syndrome and risk of coronary artery disease *(24)*. Our study, however, did not suggest any

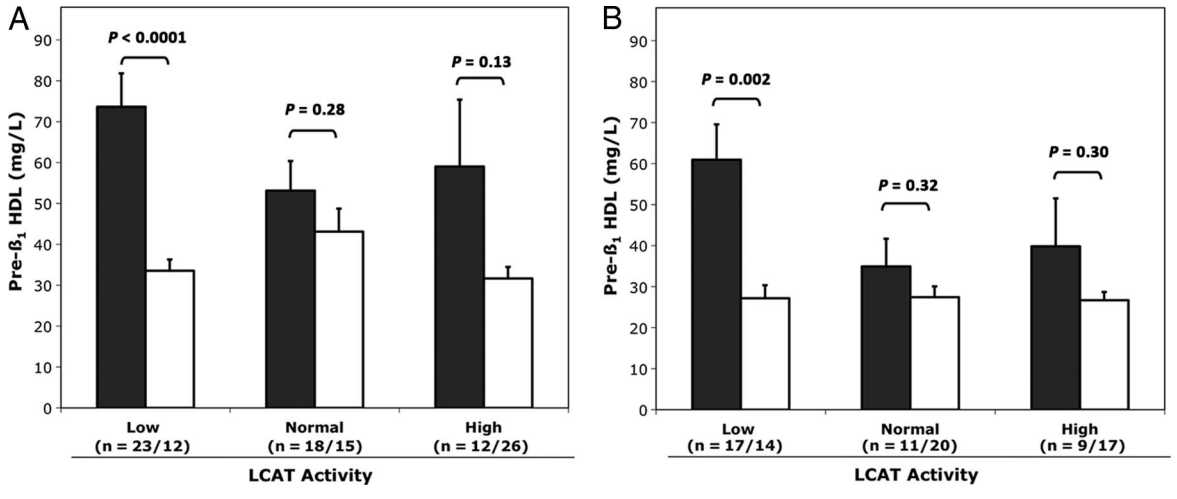

Fig. 4. Relationship between pre-β_1 HDL and LCAT.

Pre-β_1 HDL concentrations as a function of LCAT activity in tertiles in individuals with high (A) or low HDL-C concentrations (B) stratified by disease status. IHD, solid bars; no IHD, open bars. Numbers listed are for cases/controls.

role of HDL subfraction analysis as a discriminator for IHD (see online Supplemental Fig. 1). It is important to note, however, that the previous findings related to the usefulness of HDL subfractions as a diagnostic marker may not apply to our population, which was selected for either extremely high or low HDL-C values with relatively normal LDL-C and triglycerides.

One HDL subfraction that is not detected by most current HDL subfractionation methods is pre-β_1 HDL. Pre-β_1 HDL is the smallest of the 3 pre-β subfractions of HDL and is believed to represent nascent or newly formed HDL *(10, 25)*. It primarily consists of phospholipid and apoA-I and is relatively low in cholesterol compared to other HDL subfractions. Pre-β_1 HDL has been shown to be especially effective in promoting cholesterol efflux from cells by the ABCA1 transporter *(26, 27)*. It is perhaps surprising then that this HDL subfraction appeared to be positively associated with IHD (Fig. 2). It is important to realize, however, that the reverse cholesterol transport pathway, which transfers excess cellular cholesterol on HDL from the periphery to the liver for excretion *(28)*, is a cyclical pathway. The accumulation of any particular HDL subfraction may be indicative of a defect at a more distal part of the pathway. For example, patients with Tangier disease have a relative increase of pre-β_1 HDL, because of a defect in the ABCA1 transporter, which results in the accumulation of excess cholesterol in macrophages *(26)* and an increase in carotid intima-media thickness in individuals with heterozygous mutations in ABCA1 *(29)*. Unfortunately, we did not have access to fibroblasts from our participants to test for possible defects in cellular cholesterol efflux. Another known cause of increased pre-β_1 HDL is low LCAT *(30)*. LCAT mediates a key step in the reverse cholesterol transport pathway, the esterification of cholesterol that is effluxed from cells *(30)*. This process prevents the spontaneous back-diffusion of cholesterol from HDL to cells and leads to the conversion of pre-β_1 HDL to larger more lipid-rich subfractions of HDL. Consequently, as in Tangier disease, patients with LCAT deficiency have increased pre-β_1 HDL and are thought to possibly also have an increased risk of cardiovascular disease, at least in the heterozygous state *(30)*. For individuals with IHD, there was an inverse relationship between LCAT and pre-β_1 HDL, but LCAT activities did not correlate very well with pre-β_1 HDL in the control groups without disease. This finding suggests that LCAT may not be a rate-limiting step for the control group, in terms of pre-β_1 HDL formation, but that it may be for the IHD groups. Perhaps because of an unrelated defect or an imbalance in the reverse cholesterol transport pathway, more LCAT may be needed for the maturation of HDL in patients with IHD. For example, it has recently been shown that increased expression of LCAT with adenovirus in transgenic mice with mutant apoA-I results in an increase in apoA-I concentrations, lowers pre-β_1 HDL concentrations, and normalizes the dyslipidemia in these mice *(31)*.

Conflicting results have been reported for the association of pre-β_1 HDL with IHD *(32, 33)*. Increased pre-β_1 HDL concentrations have been associated with a wide variety of phenotypes normally associated with

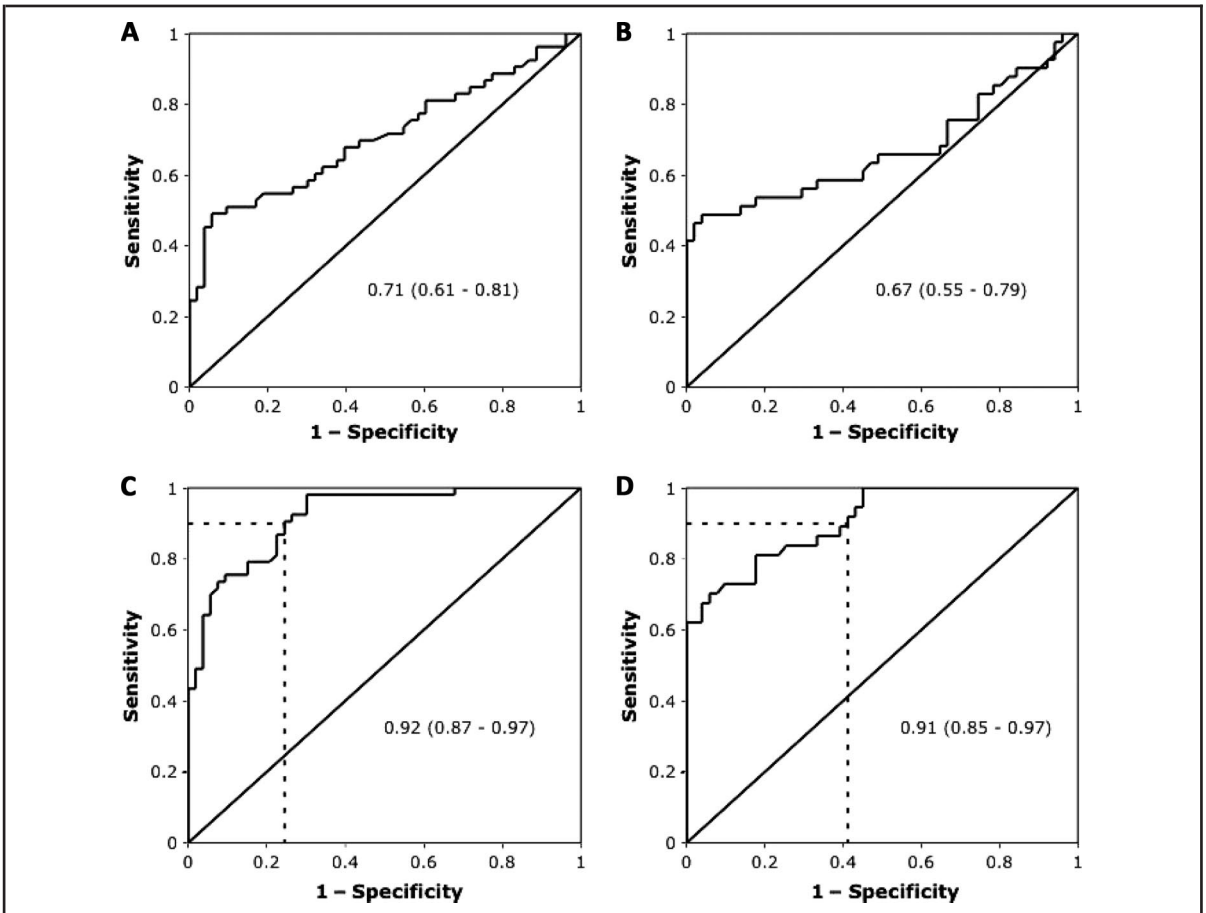

Fig. 5. Diagnostic accuracy of pre-β_1 HDL and LCAT for predicting IHD.

ROC curves for unadjusted pre-β_1 HDL (A, B) or pre-β_1 HDL concentrations adjusted for LCAT concentration (C, D) in individuals with high (left panels) or low HDL-C concentrations (right panels). The dotted lines mark the specificities for the high (75%) and low (60%) HDL-C groups at a sensitivity of 90% (C, D).

IHD, such as hypercholesterolemia *(34)* and obesity *(35)*. Other reports, however, have linked increased pre-β_1 HDL concentrations with exercise *(36)* and statin treatment *(37)*, suggesting a possible protective role of pre-β_1 HDL against IHD. Some of these apparent differences may be related to the method used for measuring pre-β_1 HDL *(32)*. Some methods, for example, do not specifically detect just pre-β_1 HDL, but also pre-β_2 or pre-β_3 HDL particles *(33)*. The ELISA method used for pre-β_1 HDL has been compared against nondenaturing 2-dimensional (2D)-gel electrophoresis, the highest HDL subfraction method currently available *(32)*. In this study, the ELISA method and 2D-gel electrophoresis showed a relatively close correlation for pre-β_1 HDL ($r = 0.833$, $P < 0.05$) *(18)*.

In addition to the analytical problems mentioned above, multiple factors have been suggested in several reports that may control changes in pre-β_1 HDL concentrations and LCAT levels and may vary in different populations and conditions. Such factors may also account for the different outcomes. One previous study of 20 patients with angiographically confirmed IHD found by 2D-gel electrophoresis that pre-β_1 HDL was increased in patients with IHD *(38)*. The analysis of LCAT activity in a subgroup of 13 patients also showed, similarly to the current study, that pre-β_1 HDL was increased in patients with IHD, who also had low LCAT activities *(38)*. Recently, however, a small increase of approximately 10% in LCAT activity has been reported to be associated with preclinical atherosclerosis in patients with metabolic syndrome *(39)*. A different assay was used for measuring LCAT activity than what was used in this study, but differences in the patient population may also account for the different association of IHD with LCAT. Given the contradictory data from animal studies on the antiatherogenic func-

tion of LCAT *(30)*, the effect of LCAT in humans may differ depending on the metabolic state, diet, and other factors that modulate lipoprotein metabolism. Furthermore, a compensatory increase in LCAT activity could be associated with atherosclerosis but may, nevertheless, still be beneficial in reducing atherosclerosis. The current study differs from most of the previous studies of pre-β_1 HDL in terms of population studied and the inclusion of other HDL parameters, such as LCAT, but supports the concept that increased pre-β_1 HDL may be an indicator of a defect in the reverse cholesterol transport pathway, which may then lead to an increased incidence of IHD.

LIMITATIONS

There are several limitations to the present study. First, it is a relatively small case-control study, and thus the results must be interpreted with caution, especially the LCAT tertiles, owing to the low numbers. It should, however, be emphasized that the low number of individuals is due to the rarity of the types of patients examined. We screened more than 2000 patients with IHD before we identified at least 50 individuals in each of the 4 groups (Fig. 1). In the future, it will be important to also examine how well pre-β_1 HDL and LCAT perform as diagnostic markers in larger populations, with HDL-C concentrations within reference intervals, to determine if these tests can be more widely used. It would also be useful to examine whether the use of these 2 tests leads to the reclassification of patients at risk for IHD in a prospective study, similar to what has been done for C-reactive protein *(40)*. Another limitation of the study was that the assay used for measuring LCAT was not suited for routine clinical testing; however, nonisotopic activity tests for LCAT are available. Many of the assays for pre-β_1 HDL are also difficult to perform and impractical for routine testing, although the ELISA used in this study can be readily performed by most clinical laboratories *(41)*. Because of the limiting amount of plasma available, HDL composition analysis was performed on supernatants after dextran-sulfate precipitation. This method may, however, also precipitate large HDL particles, which are more abundant in the high HDL-C individuals and could have affected the results of the lipid and protein composition study.

PERSPECTIVE

Low HDL-C may occur in up to 30% of the population *(42)*. Many of these patients with no other risk factors would not be considered at significant risk for IHD based on current National Cholesterol Education Program guidelines *(14)* and would likely not receive any treatment. Based on the ROC analysis performed in this study, the measurement of pre-β_1 HDL and LCAT as ancillary tests could be useful for identifying the subset of low HDL-C patients that are at risk for IHD. Although relatively rare, individuals with high HDL-C with no other risk factors but with a strong family history of IHD may also benefit from measurement of pre-β_1 HDL and LCAT. Given the complexity of HDL metabolism and composition, it is perhaps not surprising that no single feature of HDL is sufficient to fully capture all of its antiatherogenic properties. We hope that in the future, tests for HDL biomarkers such as pre-β_1 HDL and LCAT will increase our ability to predict IHD risk and lead to the development of new and better drugs that modulate HDL-C concentrations.

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