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Increased apolipoprotein E level and reduced high-density lipoprotein mean particle size associate with low high-density lipoprotein cholesterol and features of metabolic syndrome

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Abstract

The metabolic syndrome (MetS) pandemic predisposes patients to low high-density lipoprotein cholesterol (HDL-C). To successfully treat low HDL-C, there is an urgent need for a better understanding of the changes in HDL particles in the low–HDL-C state. Especially, apolipoprotein (apo) E metabolism in HDL particles is an emerging and important issue. Therefore, we determined HDL subspecies, apo E distribution, and the impact of the MetS in subjects with low and high HDL-C. We studied 246 subjects derived from the Finnish Health 2000 Health Examination Survey. The 2 groups included 113 low–HDL-C (≤10th percentile) and 133 high–HDL-C (≥90th percentile) subjects. The low–HDL-C subjects had higher apo E concentration (39.4 ± 19.4 vs 25.6 ± 8.0 μg/mL, $P < .001$) and smaller HDL mean particle size (9.0 ± 0.2 vs 9.8 ± 0.3 nm, $P < .001$). The distribution of apo E genetic isoforms could not explain the difference. Apolipoprotein E content of very low-density lipoprotein particles was comparable between the study groups. In the low–HDL-C subjects, apo E level in large HDL particles was lower ($P < .001$) compared with that in the high–HDL-C subjects. The subjects with MetS had smaller HDL mean particle size and higher serum apo E concentration. Serum apo E concentration associated positively with different MetS markers (waist circumference, triglycerides, and glucose), whereas HDL mean particle size associated with those negatively. Our results highlight that, in the low–HDL-C state, there are changes in the size and composition of HDL particles associating with MetS. Apolipoprotein E, although generally considered antiatherogenic, associates with MetS and low HDL-C. Our results emphasize the need for a better understanding of apo E metabolism in HDL particles.

1. Introduction

A low level of high-density lipoprotein cholesterol (HDL-C) is an independent, powerful risk factor for cardiovascular disease [1], even in patients with effective statin therapy and a very low level of low-density lipoprotein cholesterol (LDL-C) [2].

A key antiatherogenic mechanism of HDL is its role in the early steps of reverse cholesterol transport, that is, cholesterol efflux from macrophage foam cells. Apolipoprotein (apo) A-I and apo E function as cholesterol acceptors and are considered antiatherogenic [3]. In normolipemia, the majority of serum apo E (more than 60%) associates with HDL, whereas in hypertriglyceridemia, 70% to 90% of apo E associates with triglyceride (TG)-rich lipoproteins (TRLs) [4]. Although apo E metabolism in TRL particles has been widely studied, less is known about the role of apo E in human HDL metabolism.

The pandemic of the metabolic syndrome (MetS) as a cardiovascular risk factor emphasizes the urgent need for efficient therapies to raise HDL-C and to enhance the antiatherogenicity of specific HDL subpopulations. The question of whether the atheroprotective potential of HDL

Each study subject gave a written informed consent before participating in the study. The samples were collected in accordance with the Declaration of Helsinki, and the ethics committees of the participating centers approved the study design.

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mainly relates to total HDL-C concentration or to certain subpopulations of HDL is a debated issue. Likewise, more information is needed on the metabolism and composition of different HDL subpopulations to identify subjects with MetS at high risk for atherosclerotic events. Especially, apo E metabolism in HDL particles is both a novel and important issue in the context of antiatherogenicity of the particles. Apolipoprotein E resides in both TRL and HDL particles and modifies their metabolic function [5].

In this study, we have explored HDL subspecies in the extreme ends of the HDL-C distribution in a community-based population cohort of Finns. We examined 113 low–HDL-C subjects and 133 high–HDL-C-subjects derived from the recent Finnish population study sample Health 2000 Health Examination Survey [6]. As the role of apo E in HDL metabolism is an emerging and important issue, a specific aim was to determine serum total apo E concentration and its distribution pattern between different lipoproteins. The study design allowed us also to explore the impact of MetS on HDL subspecies and apo E distribution in this population-based cohort.

2. Methods

2.1. Study subjects

The study subjects were participants of the Health 2000 Health Examination Survey [6]. The 2-stage stratified sample comprised 10 000 persons 18 years or older, of whom 8028 were 30 years or older. Of these, 85% participated in the health examination. We selected 376 subjects representing the extreme ends of HDL-C levels (≤10th and ≥90th sex-specific percentiles): 185 subjects with low HDL-C and 191 with high HDL-C levels. The HDL-C limits were as follows: low–HDL-C men, less than or equal to 1.03 mmol/L; low–HDL-C women, less than or equal to 1.23 mmol/L; high–HDL-C men, greater than or equal to 1.79 mmol/L; and high–HDL-C women, greater than or equal to 2.24 mmol/L. Subjects with diabetes, alcohol abuse, or malignancy were excluded. *Diabetes* was defined as a previous diabetes diagnosis, use of antidiabetic agents, or venous plasma glucose greater than or equal to 7.0 mmol/L, confirmed on a subsequent day [7]. *Alcohol abuse* was defined as greater than 160 g of alcohol per week for women and greater than 310 g of alcohol per week for men. In addition, subjects using systemic estrogen or corticosteroid therapy or other drugs affecting HDL metabolism (except statins) were excluded. Because of these criteria, 130 subjects had to be excluded. Accordingly, the final analyses included 246 subjects: 113 with low HDL-C (72 men, 41 women) and 133 with high HDL-C (75 men, 58 women). Each study subject gave a written informed consent before participating in the study. The samples were collected in accordance with the Declaration of Helsinki, and the ethics committees of the participating centers approved the study design.

The *MetS* was defined as waist of at least 94 cm in men and at least 80 cm in women plus any 2 of the following: raised TG (>1.7 mmol/L), raised blood pressure (systolic ≥130 mm Hg, diastolic ≥85 mm Hg, or treatment of previously diagnosed hypertension), or raised fasting plasma glucose (≥5.6 mmol/L), according to the International Diabetes Foundation criteria [8], but omitting HDL-C criteria.

2.2. Laboratory analyses

Venous blood samples were drawn after an overnight fast. Serum and EDTA plasma samples were stored at −70°C before analysis. Serum total cholesterol (TC), TG, and HDL-C were measured with Olympus AU400 clinical chemistry analyzer (Olympus, Hamburg, Germany) by fully enzymatic methods (Olympus kits OSR 6116 and 6133 for TC and TG, respectively, and Roche Diagnostics kit 3030024 for HDL-C [Roche Diagnostics, Mannheim, Germany]). Low-density lipoprotein cholesterol was calculated using the formula of Friedewald et al [9]. Low-density lipoprotein cholesterol was not calculated if TG was greater than 4.0 mmol/L (8 subjects from low–HDL-C group). Concentrations of apo A-I and apo B were measured with Olympus AU400 analyzer by immunoturbidimetric methods (kits 64265 and 67249 from Orion Diagnostica, Espoo, Finland). Serum apo A-II was measured with Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) immunoturbidimetrically (Wako Chemicals, Neuss, Germany, and own polyclonal antibody produced in rabbits against purified human apo A-II). Serum apo E concentration was quantitated by enzyme-linked immunosorbent assay [10]. Apolipoprotein E phenotyping was performed with isoelectric focusing and Western blotting as previously described [11]. Plasma glucose was measured by the glucose dehydrogenase method (Merck Diagnostica, Darmstadt, Germany).

High-density lipoprotein subspecies distribution and HDL mean particle size were determined with native gradient gel electrophoresis [12] with minor modifications as previously described [13]. The molecular size intervals for HDL subspecies 2b, 2a, 3a, 3b, and 3c were used according to Blanche et al [12]; and for each subspecies, the relative area under the densitometric scan is reported. High-density lipoprotein mean particle size was calculated by multiplying the mean size of each HDL subclass by its relative area under the densitometric scan [14]. Low-density lipoprotein peak particle size was measured with gradient gel electrophoresis as previously described in detail [15].

Distribution of apo E in serum samples was analyzed by high-performance liquid chromatography system (Merck-Hitachi) using size-exclusion chromatography on a Superose 6HR column (GE Healthcare, Buckinghamshire, United Kingdom). Column was equilibrated with phosphate-buffered saline, pH 7.4 containing 0.05% Tween 20 at the flow rate of 0.5 mL/min. Serum samples (400 μL) were applied on the column; and 0.5-mL fractions were collected for apo E, cholesterol, and TG measurements. Apolipoprotein E distribution for 16 low–HDL-C and 13 high–HDL-C

subjects was analyzed. The subjects of this subsample were selected randomly after excluding subjects with statin therapy or extreme hypertriglyceridemia (TG limit ≤2.7 mmol/L). Apolipoprotein E distributed in 3 positions, corresponding to very low-density lipoprotein (VLDL), large HDL, and small HDL. The fractions enriched with apo E were combined, and the apo E distribution was calculated.

Phospholipid transfer protein (PLTP) activity was measured using the radiometric assay previously described [16] with minor modifications [17]. The PLTP concentration was measured with enzyme-linked immunosorbent assay [18]. Cholesterol ester transfer protein activity was measured with radiometric assay as described by Groener et al [19].

Blood pressure values are the mean values from 3 consecutive measurements carried out in 1- to 2-minute intervals. Data on alcohol consumption and physical activity were collected from questionnaires filled in by the study subjects.

2.3. Statistical analyses

Statistical comparisons were performed with SPSS 14.0 for Windows (SPSS, Chicago, IL). Results are expressed as means ± SD for continuous variables, except for TG and alcohol consumption, which were expressed as medians and interquartile ranges because of their skewed distribution. For categorical variables, frequencies are given. Continuous variables were compared between the groups by general linear model analysis of covariance (ANCOVA) with sex and body mass index (BMI) as covariates. The statistical significance of the trend between the groups according to the number of MetS components was tested by general linear model ANCOVA (sex and BMI as covariates). $P < .05$ was considered significant (2-tailed). Frequency distribution of categorical variables was compared between the groups with the χ^2 test. Correlations were calculated with partial Pearson correlation analysis (controlling for the effect of sex). Variables with skewed distribution were $\log_{10}$ transformed before analysis; but values given in text, tables, and figures are nontransformed.

3. Results

Table 1 shows the clinical and biochemical characteristics of the study subjects. Of the low–HDL-C subjects, 64% were men, whereas 56% of the high–HDL-C subjects were men. The sex distribution was similar in both groups ($P = .243$). Blood pressure values were similar in the groups (Table 1), but the history of hypertension was more common among the low–HDL-C subjects (38% vs 20%, $P = .001$). Coronary heart disease was more common in the low–HDL-C subjects (17% vs 5%, $P = .001$), but there was no significant difference in the amount of statin users (15% vs 8%, $P = .060$). Total cholesterol concentration was higher in the high–HDL-C group because of the higher HDL-C level in the face of comparable LDL-C. Low HDL-C is often presented together with higher TGs, and this was shown also here as higher TG level in low–HDL-C group (Table 1).

Table 1
Clinical and biochemical characteristics of the study subjects

	Low–HDL-C subjects	High–HDL-C subjects	*P*
n (men/women)	113 (72/41)	133 (75/58)	
Age, y	59 ± 8	58 ± 8	.422
BMI, kg/m^2	29.9 ± 5.2	24.8 ± 3.6	<.001
Waist, cm	100.5 ± 12.4	87.6 ± 11.3	<.001
Systolic blood pressure, mm Hg	139 ± 20	138 ± 21	.156
Diastolic blood pressure, mm Hg	85 ± 10	84 ± 10	.200
HDL-C, mmol/L	0.97 ± 0.13	2.24 ± 0.28	<.001
TG, mmol/L	2.00 (1.40-2.80)	0.80 (0.60-1.00)	<.001
Glucose, mmol/L	6.0 ± 0.8	5.6 ± 0.6	.005
TC, mmol/L	5.50 ± 1.13	5.87 ± 0.83	<.001
LDL-C, mmol/L	3.52 ± 0.97	3.23 ± 0.78	.270
Apo A-I, mg/dL	137.5 ± 19.6	211.6 ± 23.6	<.001
Apo A-II, mg/dL	32.1 ± 6.2	40.0 ± 9.6	<.001
Apo B, mg/dL	133.3 ± 27.8	103.8 ± 21.0	<.001
Apo E, μg/mL	39.4 ± 19.4	25.6 ± 8.0	<.001
HDL mean particle size, nm	9.0 ± 0.2	9.8 ± 0.3	<.001
LDL peak particle size, nm	24.8 ± 1.4	27.2 ± 0.8	<.001
Current smoking, n (%)	21 (19%)	31 (23%)	.228
Alcohol consumption, g/wk	23 (0-69)	51 (11-125)	<.001
Exercise ≥2×/wk, n (%)	59 (52%)	95 (71%)	.002

Values are means ± SD, except for TG and alcohol consumption, where median and interquartile values are given.

The low–HDL-C subjects displayed smaller HDL mean particle size (Table 1). This was mainly caused by the marked reduction in the proportion of large HDL_{2b} particles; the low–HDL-C subjects had only one third of the HDL_{2b} proportion observed in the high–HDL-C subjects (Fig. 1). Low-density lipoprotein peak particle size was clearly smaller in the low–HDL-C subjects despite the comparable LDL-C level (Table 1). Phospholipid transfer protein mass was lower in the low–HDL-C subjects (7.79 ± 2.28 vs 8.55 ± 2.88 μg/mL, $P = .042$), but there were no statistically significant differences in PLTP activities (data not shown). A nonsignificant trend in cholesterol ester transfer protein activity toward lower activity in the low–HDL-C subjects was observed (28.9 ± 6.7 vs 31.4 ± 7.3 nmol/[mL h], $P = .052$).

Serum apo E concentration was significantly higher (1.5-fold) in the low–HDL-C subjects (Table 1). Each apo E phenotype was associated with a higher apo E concentration in the low–HDL-C group compared with the high–HDL-C group (Table 2). In the low–HDL-C subjects, apo E level in large HDL particles was lower compared with that in the high–HDL-C subjects (Fig. 2). Apolipoprotein E concentration in VLDL fraction did not differ between the study groups. After adjusting for sex, the small difference in apo E concentration in small HDL particles was not statistically significant (Fig. 2).

Metabolic syndrome was present in 66% of the low–HDL-C and in 20% of the high–HDL-C subjects ($P < .001$).

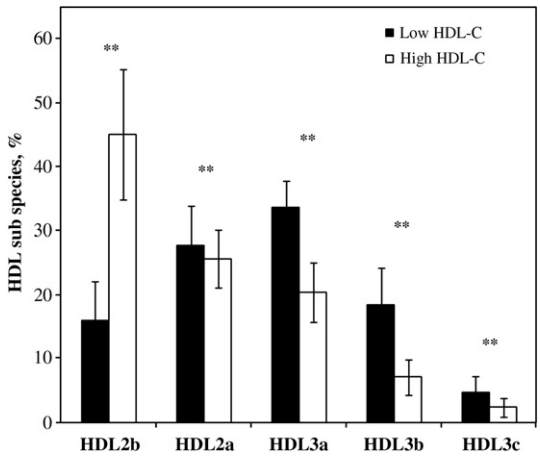

Fig. 1. The percentages of HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c} in the low–HDL-C and high–HDL-C subjects. $^{**}P < .001$ between the low– and high–HDL-C groups (general linear model ANCOVA; sex and BMI as covariates).

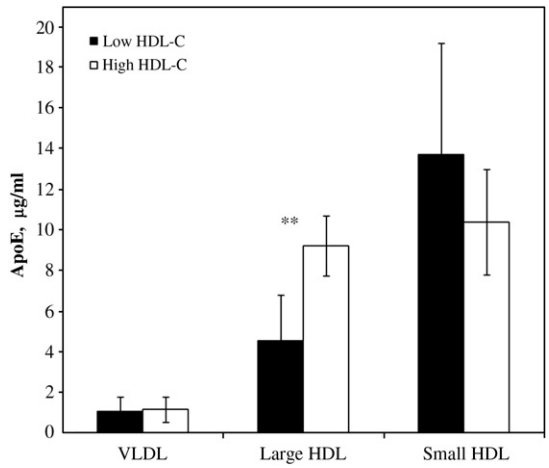

Fig. 2. Size-exclusion chromatography elution profiles of serum apo E in high–HDL-C (n = 16) and low–HDL-C (n = 13) subjects. Apolipoprotein E distributed in 3 positions, corresponding to VLDL, large HDL, and small HDL. $^{**}P < .001$ between the low– and high–HDL-C groups (general linear model ANCOVA; sex and BMI as covariates).

We next allocated the low–HDL-C subjects to those with and without MetS. The subjects fulfilling the MetS criteria had lower percentage of HDL_{2b} particles and smaller HDL mean particle size than those without MetS (Table 3). Apolipoprotein E level was higher in the subjects with MetS (Table 3). Similar trends were observed in the high–HDL-C group (Table 3).

To further clarify the impact of MetS on HDL subclass distribution and apo E concentration, we divided the low–HDL-C subjects into 4 groups according to the number of MetS components they had (in addition to low HDL-C). Interestingly, the more MetS components the subjects had, the lower were the proportions of HDL_{2b} particles (Fig. 3A), and the smaller was HDL mean particle size (Fig. 3C). In contrast, serum apo E concentration displayed an opposite trend; apo E levels increased parallel with the number of MetS components (Fig. 3E). Similar trends were observed in the high–HDL-C subjects (Fig. 3B, D, and F, accordingly). Apolipoprotein E correlated positively with waist circumference, TG, and glucose, whereas HDL mean particle size showed an opposite trend with waist circumference and TG (Table 4).

As 15% of low–HDL-C subjects and 8% of high–HDL-C subjects were on statin therapy, we repeated all the analysis excluding subjects with statin therapy; but this did not change the results (data not shown).

4. Discussion

The pandemic of MetS and obesity is worldwide and predisposes a rapidly growing number of patients to low HDL-C. The absence of efficient and well-tolerated HDL-raising therapies clearly indicates the need for a better understanding of the complexity of HDL metabolism.

Table 2
Apolipoprotein E concentration according to apo E phenotype distribution

	Low–HDL-C group		High–HDL-C group	
n	101		115	
Apo E phenotype[a]	n (%)	Serum apo E, µg/mL	n (%)	Serum apo E, µg/mL
2-2	1 (1%)	57.3	–	–
3-2	2 (2%)	35.9 ± 1.49	7 (5%)	27.8 ± 6.2
3-3	54 (48%)*	41.7 ± 16.4	79 (59%)	27.9 ± 7.4
4-2	3 (3%)	35.7 ± 4.9	–	–
4-3	29 (26%)	39.0 ± 27.4	23 (17%)	21.4 ± 6.1
4-4	12 (11%)	32.3 ± 11.2	6 (5%)	19.3 ± 4.3

[a] Data missing from 12 low–HDL-C and 18 high–HDL-C subjects.
* $P < .05$ between low–HDL-C and high–HDL-C groups.

Table 3
High-density lipoprotein cholesterol concentration, HDL_{2b} percentage, HDL mean particle size, and apo E concentration in subjects with and without MetS

	MetS subjects	MetS-free subjects	*P*
Low–HDL-C group, n (men/women)	75 (46/29)	35 (23/12)	
HDL-C, mmol/L	0.95 ± 0.12	1.01 ± 0.10	.007
HDL_{2b}, %	14.6 ± 5.7	18.9 ± 6.0	.001
HDL mean particle size, nm	9.0 ± 0.2	9.1 ± 0.2	.001
Apo E, µg/mL	44.2 ± 21.8	30.4 ± 8.8	<.001
Statin therapy, n (%)	13 (17%)	4 (11%)	.425
High–HDL-C group, n (men/women)	26 (20/6)	105 (53/52)	
HDL-C, mmol/L	2.14 ± 0.29	2.27 ± 0.27	.702
HDL_{2b}, %	41.5 ± 8.8	46.2 ± 9.9	.445
HDL mean particle size, nm	9.8 ± 0.3	9.9 ± 0.3	.451
Apo E, µg/mL	30.5 ± 7.2	24.8 ± 7.7	.063
Statin therapy, n (%)	1 (4%)	9 (9%)	.417

Values are means ± SD. Metabolic syndrome classification could not be done for 3 low–HDL-C and 2 high–HDL-C subjects because of missing data.

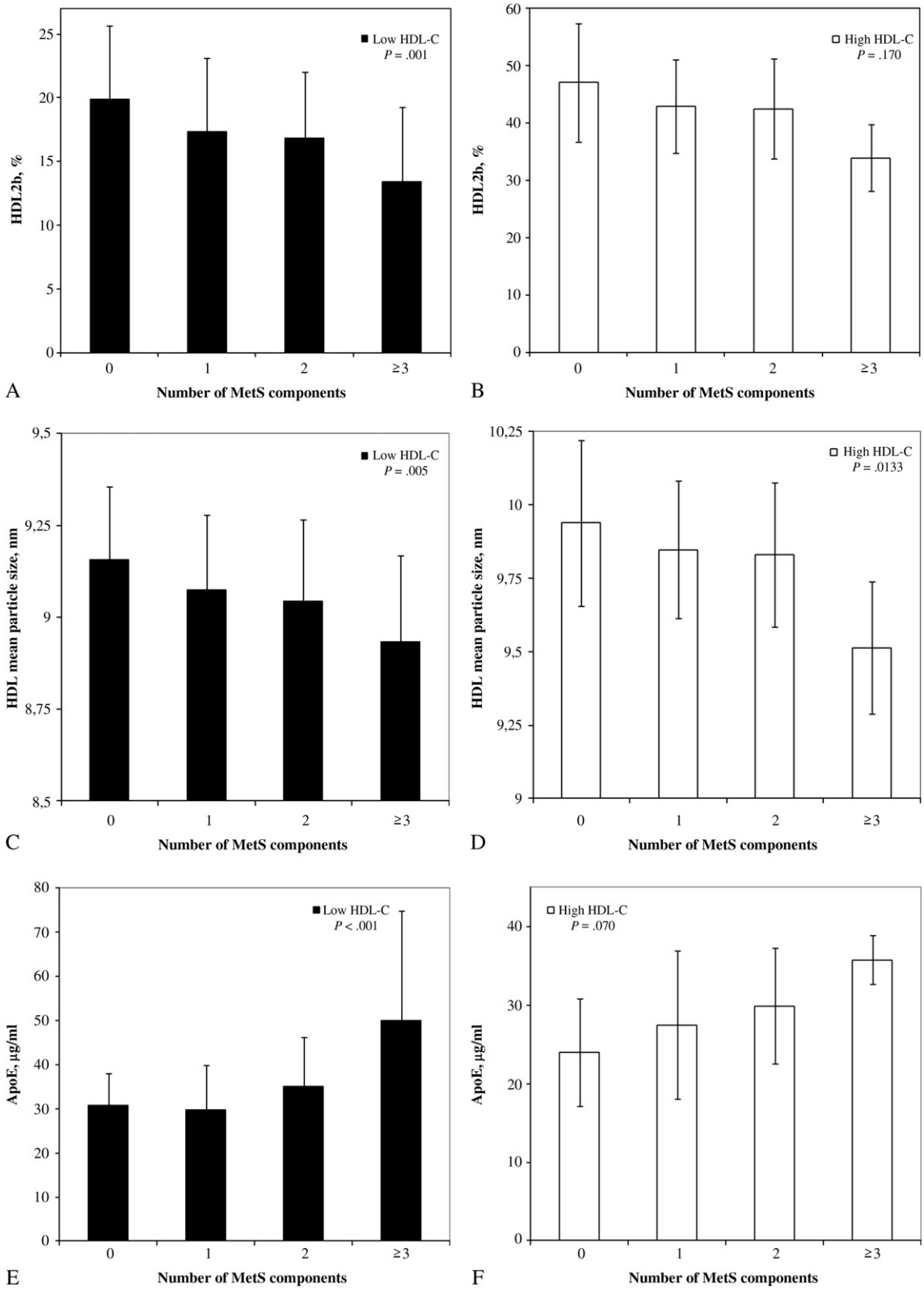

Fig. 3. High-density lipoprotein $_{2b}$ percentage (A, B), HDL mean particle size (C, D), and concentration of apo E (E, F) as a function of the number of MetS components. *P* value indicates the significance of the trend between the MetS groups in low–HDL-C and high–HDL-C subjects. According to the International Diabetes Foundation criteria, if the waist circumference criterion (>94 cm in men, >80 cm in women) was absent, the subject was classified to have no MetS components. The statistical significance of the trend between the groups according to the number of MetS components was tested by general linear model ANCOVA (sex and BMI as covariates).

Table 4
Spearman partial correlations (controlling for sex) of HDL mean particle size and apo E concentration between the markers of the MetS

	Low–HDL-C subjects		High–HDL-C subjects	
n	113		133	
	r	*P*	*r*	*P*
HDL mean particle size				
Waist circumference	−0.206	.042	−0.126	.163
TGs	−0.286	.002	−0.055	.534
Glucose	−0.156	.100	−0.039	.658
Apo E concentration				
Waist circumference	0.208	.049	0.300	.001
TGs	0.756	<.001	0.392	<.001
Glucose	0.490	<.001	−0.030	.749

Especially, apo E metabolism in the low–HDL-C state is an emerging and important issue. The impact of apo E in the pathogenesis of atherosclerosis has been clearly demonstrated in animal models [20,21]. It has been demonstrated that apo E plays a major role in the lipid efflux from macrophage foam cells [22] and is generally considered antiatherogenic. However, the role of apo E in human atherosclerosis is far from resolved. Especially, little is known about the role of apo E in human HDL metabolism.

We report here the apo E concentration and subclass distribution of HDL in the low–HDL-C subjects compared with the high–HDL-C subjects, representing the extreme ends of the HDL-C distribution in a population sample of Finns. To the best of our knowledge, this is the first study to describe apo E concentration and phenotype together with HDL subspecies distribution in carefully characterized low– vs high–HDL-C subjects. The comparison of low– vs high–HDL-C subjects in a population-based study material allows us to study maximal differences and the impact of MetS on lipoprotein species. We describe a clear-cut effect of escalating features of the MetS on HDL subspecies, HDL mean particle size, and serum apo E concentration and distribution.

Our interesting observation that the low–HDL-C subjects have significantly higher serum apo E levels than the high–HDL-C subjects may help to explain the role of apo E in HDL metabolism. The difference in apo E concentration between the 2 HDL-C groups was not explained by differences in the distribution of genetic isoforms of apo E. Subjects with each apo E phenotype in the low–HDL-C group had higher apo E concentration than the subjects sharing the same phenotype in the high–HDL-C group. In addition, the ε-4 allele was more frequent in the low–HDL-C subjects, who had higher serum apo E levels. Earlier, the ε-4 allele has been associated with low apo E concentrations [23]. Statin therapy has been suggested to decrease plasma apo E levels [24]; but in the present study, apo E levels remained unchanged after the exclusion of statin users.

We analyzed the apo E distribution in the low– and high–HDL-C subjects. The gel filtration experiments demonstrated, first, that VLDL particles did not contain excess amounts of apo E and, second, that apo E eluted in 2 locations, both in the position of large HDL particles and in the small HDL. These results agree well with those reported by Krimbou et al [25].

Large HDL particles of our low–HDL-C subjects had less apo E than those of the high–HDL-C subjects. Correspondingly, acute myocardial infarction patients with lower HDL-C had a reduction in large apo E–containing HDL particles [26]. The reduction of apo E in large HDL particles may have an impact on the development of atherosclerosis because apo E–enriched large HDL particles have enhanced ability to promote cholesterol efflux from foam cells [27].

More evidence on the importance of apo E distribution between different HDL subfractions comes from the study where cardiovascular patients had apo E–enriched HDL_3, although there was no difference in serum HDL-C [28]. Furthermore, the same group reported recently that combined niacin-statin therapy lowered HDL_3-associated apo E [29]. To exclude the effect of statins on apo E metabolism in VLDL [24] and HDL [28] fractions, we chose only subjects without statin therapy for the apo E distribution subanalysis.

We have shown earlier that apo E could function as an activator for PLTP [30]. Here we report that PLTP mass was higher among the high–HDL-C subjects, with no differences in PLTP activity. This means that PLTP specific activity was lower in the high–HDL-C subjects who also had larger HDL mean particle size. This observation agrees well with the recent study [31] that reported a significant inverse relationship between HDL particle size and plasma PLTP specific activity. This implies that HDL particle volume may modulate PLTP specific activity in the vascular compartment and that one factor could be the content of apo E in large HDL particles. Taken together, the observed different concentrations of apo E between the high– and low–HDL-C subjects in the HDL subpopulations are of great interest and need further studies to explain potential clinical relevance with respect to atherosclerosis.

Higher serum apo E concentration has been associated with MetS [23,32], in accordance with our results. We report that serum apo E concentration rises and HDL mean particle size diminishes with the increasing number of MetS components. This is the first study in low–HDL-C subjects to simultaneously report significant correlations of apo E and HDL mean particle size with the markers of MetS: waist circumference, TGs, and glucose.

The smaller HDL mean particle size in low–HDL-C subjects supports the view that reduction in HDL particle size is an additional feature of the atherogenic high-TG/low–HDL-C profile very often associated with abdominal obesity, MetS, and type 2 diabetes mellitus [33]. The smaller HDL mean particle size was due to a major 60% to 70% difference in the proportion of large HDL_{2b} particles as compared with the high–HDL-C subjects. The reduction of large HDL particles in the low–HDL-C state

is in good accordance with our previous study in subjects with familial low HDL-C [34], as well as with other studies [35,36].

The study design comparing the extreme ends of HDL-C distribution in the population sample may be argued as a potential limitation of the study. However, we intentionally chose this approach to study maximal differences and minimize the effect of confounding factors. We consider that the study subjects here represent well the Finnish low– and high–HDL-C population, as the Health 2000 Health Examination Survey is a comprehensive sample of the Finnish population.

The study, however, raised further mechanistic questions. Although generally considered antiatherogenic, apo E was higher in the low–HDL-C subjects and associated with the MetS. Whether the rise in plasma apo E is a compensatory response or a marker of the proatherogenic low–HDL-C state remains to be solved. Therefore, mechanistic/functional studies are needed to get a better insight of the apo E metabolism in the low–HDL-C state and its relation to atherosclerosis in humans.

Given that sex is generally known to affect lipoprotein metabolism, results on the effect of sex on plasma apo E levels are however inconclusive [37,38]. To minimize the confounding effect of sex, we used sex as a covariate in the statistical analysis.

In conclusion, we confirm in this population-based cohort that the subjects with low HDL-C have higher apo E concentration than the subjects with high HDL-C. The distribution of apo E phenotypes did not explain the difference. Large HDL particles of the low–HDL-C subjects had a decreased apo E content. Whether the changes in HDL apo E distribution influence atherogenesis remains to be determined. In MetS subjects, we report a reduction of large HDL_{2b} particles, smaller HDL mean particle size, and higher apo E concentration. Smaller HDL particle size and higher apo E level associated with the several components of the MetS: waist circumference, glucose, and TGs.

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