Postheparin plasma lipoprotein and hepatic lipase are determinants of hypo- and hyperalphalipoproteinemia

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Abstract To study the role of the two postheparin plasma lipolytic enzymes, lipoprotein lipase (LPL) and hepatic lipase (HL) in high density lipoprotein (HDL) metabolism at a population level, we determined serum lipoproteins, apoproteins A-I, A-II, B, and E, and postheparin plasma LPL and HL activities in 65 subjects with a mean HDL-cholesterol of 34 mg/dl and in 62 subjects with a mean HDL-cholesterol of 87 mg/dl. These two groups represented the highest and lowest 1.4 percentile of a random sample consisting 4,970 subjects. The variation in HDL level was due to a 4.1-fold difference in the HDL_2 cholesterol ($P < 0.001$) whereas the HDL_3 cholesterol level was increased only by 32% ($P < 0.001$) in the group with high HDL-cholesterol. Serum apoA-levels were 128 ± 2.2 mg/dl and 210 ± 2.8 mg/dl (mean ± SEM) in hypo- and hyper-HDL cholesterolemia, respectively. Serum apoA-II concentration was elevated by 28% ($P < 0.001$) in hyperalphalipoproteinemia. The apoA-I/A-II ratio was elevated only in women with high HDL-cholesterol but not in men, suggesting that elevation of apoA-I is involved in hyperalphalipoproteinemia in females, whereas both apoA proteins are elevated in men with high HDL cholesterol. Serum concentration of apoE and its phenotype distribution were similar in the two groups. The HL activity was reduced in the high HDL-cholesterol group (21.2 ± 1.5 vs. 38.5 ± 1.8 μmol/h/ml, $P < 0.001$), whereas the LPL activity was elevated in the group with high HDL-cholesterol compared to subjects with low HDL-cholesterol (27.8 ± 1.3 vs. 19.9 ± 0.8 μmol/h/ml, $P < 0.001$). The HL and LPL activities correlated in opposing ways with the HDL_2 cholesterol ($r = 0.57$, $P < 0.001$ and $r = 0.51$, $P < 0.001$, respectively), and this appeared to be independent of the relative ponderosity by multiple correlation analysis. The results demonstrate major influence of both HL and LPL on serum HDL cholesterol concentration at a population level. — **Kuusi, T., C. Ehnholm, J. Viikari, R. Härkönen, E. Vartiainen, P. Puska, and M-R. Taskinen.** Postheparin plasma lipoprotein and hepatic lipase are determinants of hypo- and hyperalphalipoproteinemia. *J. Lipid Res.* 1989. **30:** 1117–1126.

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The HDL lipoprotein class, the density 1.063–1.21 g/ml interval, is a heterogeneous mixture of lipoprotein particles containing two major subfractions, HDL_2 and HDL_3 (1). The larger, less dense HDL_2 is the main determinant of the total HDL cholesterol concentration while the smaller HDL_3 particles vary only within narrow limits. The antiatherogenic property of HDL is supposed to depend on its ability to take up tissue cholesterol and transfer it to the liver for excretion. The addition of lipids into HDL results in a conversion of HDL_3 to HDL_2 (2, 3) and thus elevated HDL_2 is proposed to reflect the anti-atherogenic activity of HDL. Accordingly, a negative relationship has been observed between the HDL_2 cholesterol concentrations and the extent of coronary artery disease in several studies (4, 5).

The HDL_2 particles are formed following the LPL-catalyzed hydrolysis of VLDL triglycerides when apoproteins and surface phospholipids released from VLDL fuse with pre-existing HDL_3 particles (2,3,6). The core of the surface-enriched HDL particles is filled with cholesterol collected from peripheral cells and esterified by lecithin:cholesterol acyltransferase (LCAT) (1). The formation of HDL (HDL_2) depends on the activity of lipoprotein lipase (LPL) and it is promoted under conditions of high VLDL turnover (7). Thus, a positive correlation has been demonstrated between postheparin plasma LPL activity and serum HDL_2 cholesterol concentration under several clinical conditions (6–9).

The degradation of HDL (HDL_2) particles is enhanced by another lipolytic enzyme, hepatic lipase (HL) (10, 11), which catalyzes the hydrolysis of HDL phospholipids and

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; RID, radial immunodiffusion; BMI, body mass index.

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triglycerides (12–14). In experimental animals the inactivation of HL by specific antiserum increases the level of HDL_2 cholesterol (15–17). Accordingly, serum HDL_2 level is elevated in HL deficiency states in man (18–20). The two enzymes, LPL and HL, exert opposing effects on the metabolism of plasma HDL (6). In addition to lipolytic enzymes that regulate the lipid content of HDL particles, the level of HDL is modulated by its apoproteins A-I, A-II, C, and E (1). Apoprotein A-I displays genetic variation which is likely to influence the HDL level (21). Variation of HDL (HDL_2) levels could be related also to serum apoE levels or apoE phenotypes. However, few studies have addressed the impact of these apoprotein characteristics or that of lipolytic enzymes on serum HDL cholesterol at a population level (22, 23).

The present study was conducted to determine HDL and its subfractions, apoprotein variants, and postheparin lipolytic enzyme activities in subjects with hypo- and hyperalphalipoproteinemia. The hypo-HDL subjects had a mean HDL cholesterol of 34 mg/dl whereas the hyper-HDL subjects had mean HDL cholesterol of 87 mg/dl. The 127 subjects studied represented the highest and lowest 1.4 percentiles of a population sample of 4,970 subjects previously screened for CHD risk factors including also HDL cholesterol.

MATERIALS AND METHODS

Subjects

Subjects belonging to the highest 1.4 percentile or lowest 1.4 percentile HDL cholesterol in a population sample (n = 70 in both groups) were invited by a letter to the present study. The initial random population sample of 4,970 Finns was a subset of a WHO MONICA (Monitoring of Cardiovascular risk factors) study and had been screened 1 year earlier for HDL cholesterol by the dextran-sulfate precipitation method (24). Sixty-two subjects were living in the surroundings of and in Turku city in southwestern Finland and were considered as urban Finns. The remaining 65 subjects were from the area of Joensuu and represent a rural Finnish population. Thirty-two and 33 subjects in the low HDL group were from these two areas, respectively. In the high HDL group, corresponding numbers were 30 and 32. The two 70-subject groups were computer-selected as having the highest and lowest HDL-cholesterol values among the subjects living in these areas registered in the MONICA data file in the Digital Vax 11/750 computer of the National Public Health Institute of Finland. The low HDL group (n = 65) consisted of 11 women and 54 men with mean (± SEM) body mass index (weight/height^2) of 28.8 ± 1.72 (range 23.1–42.8) and 28.7 ± 0.53 (range 19.5–39.4), respectively. The high HDL group (n = 62) contained 49 women with a mean body mass index 24.2 ± 0.49 (range 19.1–35.4) and 13 men with a body weight of 23.6 ± 0.88 (range 15.8–27.7). The ages ranged between 19 and 64 yr (48.1 ± 1.54 yr) in the low HDL group and between 25 and 64 yr (47.2 ± 1.45) in the high HDL group. The prevalences of hypertension, lipoprotein abnormalities, diabetes, and smoking and the use of drugs affecting plasma lipids are given in **Table 1**.

Plasma lipoproteins

Blood was collected at 7:30 AM after an overnight (from 8:00 PM) fast, and serum was separated by centrifugation at 3000 rpm for 10 min at 4°C. Lipoproteins were separated in a Beckman L7-70 ultracentrifuge (Beckman Instruments, Palo Alto, CA) using a Kontron TZT 45.6 rotor (Kontron AG, Basel, Switzerland) (25). A running time of 18 h at 38,000 rpm at a density of 1.006 g/ml was used for the isolation of VLDL, 24 h at 42,000 rpm at 1.063 g/ml for the isolation of LDL, and 48 h at 42,000 rpm at 1.125 g/ml for the isolation of HDL_2. The bottom fraction of density 1.125 g/ml was taken to represent the HDL_3 lipids. Recoveries of cholesterol and triglycerides in the four lipoprotein fractions were 90 ± 3% (± SEM) and 88 ± 4%. The cholesterol concentration in the LDL bottom fraction was routinely measured, and the recovery of HDL_2 and HDL_3 cholesterol with respect to LDL bottom fraction cholesterol was 89 ± 3%.

Rate zonal separation of HDL subfractions was performed by the method of Groot et al. (26) using the Beckman Ti SW-40 rotor operated at 41,000 rpm in the Beckman L7-70 ultracentrifuge for 21 h at 4°C. The tubes were fractionated and the absorbance at 280 nm (OD 280 nm) was registered with an LKB Uvicord II detector.

TABLE 1. Presence of hypertension, lipoprotein abnormalities, diabetes, smoking, and drugs affecting plasma lipids in the high-HDL and low-HDL subjects and in subjects with previous myocardial infarction (MI)

	High-HDL (n = 62)	Low-HDL (n = 65)	MI-Patients (n = 13)
Hypertension	4	12	6
Lipoprotein phenotype			
Normal	49	17	2
II A	3	9	1
II B	3	15	4
IV	7	24	6
Diabetes mellitus	0	3	1
Smoking prevalence	14	21	3
Medication			
Beta blockers	2	10	4
Estrogens/progestins	4	0	0
Thiazides	4	2	1

Cut-off points: Normals: LDL-C < 174 mg/dl, serum triglycerides ≤ 177 mg/dl; II A: LDL-C > 174 mg/dl, serum triglycerides ≤ 177 mg/dl; II B: LDL-C > 174 mg/dl, serum triglycerides > 177 mg/dl; IV: LDL-C < 174 mg/dl, serum triglycerides > 177 mg/dl.

Apolipoproteins A-I and A-II were determined by radial immunodiffusion (RID) using purified apoA-I and apoA-II as standards (27). Each assay included three control sera, with low, medium, and high respective apoprotein concentrations. The assays had been checked with control sera kindly provided by Drs. Cheung and Albers, University of Washington, Seattle, WA. The interassay coefficients of variation (CV) of the apoA-I and A-II methods were 4.3% and 5.3% respectively. ApoB was determined by RID using commercial plates obtained from Behring (Behring GmbH, FRG) calibrated with the standards and control sera according to the manufacturer's instructions. The CV of this method is 4.7%. Serum apoE concentration was determined by radioimmunoassay kindly performed in the Gladstone Research Laboratories by Dr. R. W. Mahley (28). The apoE phenotyping was carried out by isoelectric focusing (29). ApoA phenotyping was performed according to the method of Menzel, Kladetsky, and Assmann (30).

Assay of lipase activities

After an overnight fast, postheparin blood was drawn into ice-cold heparinized tubes 5 and 15 min after an intravenous injection of 100 IU of heparin per kg of body weight. Plasma was separated at 4°C and stored frozen until assayed. Postheparin plasma LPL and HL activities were determined by an immunochemical method (31) using gum arabic-stabilized tri[1-^{14}C]oleoylglycerol as a substrate. The LPL activity was determined, after inhibition of the HL activity by a specific antiserum, using 3.2 mmol/l tri[1-^{14}C]oleoylglycerol at 0.1 mol/l NaCl with 10% normal human serum as activator. The HL activity was determined at 1.0 mol/l NaCl in the absence of serum. The lipase activities are expressed as μmol FFA released in 1 h per ml of postheparin plasma. Each assay series contained two standard sera.

Other methods

Serum total and lipoprotein cholesterol and triglyceride concentrations were determined enzymatically using Boehringer kits No. 263691 and 297771, respectively, in a Kone Olli-C Analyzer (Kone Ltd., Espoo, Finland).

Statistical analyses

The statistical treatment of the data was performed in a Digital Rainbow computer operated with the NWA software (Northwestern Analytical Inc, Portland, OR). Logarithmic transformation of the data was performed for parameters that were not normally distributed on the basis of significant skewness of distribution. This was necessary in case of serum total and VLDL-triglycerides and VLDL-cholesterol. The differences among the four HDL-groups (the high-HDL and low-HDL, male and female), and the various apoE phenotype groups were tested by one-way analysis of variance. The relationships between the different parameters were evaluated by simple and multiple regression analysis.

RESULTS

The means of HDL cholesterol concentration (± SEM) averaged 34 ± 1 mg/dl in the low-HDL group and 87 ± 2 mg/dl in the high-HDL group (**Table 2**). The variation of HDL cholesterol was mainly due to a 4.1-fold difference in the HDL_2 fraction whereas the mean concentration of HDL_3 cholesterol was, albeit significantly, only 32% higher in the high-HDL group than in the low-HDL group. Similarly, rate zonal centrifugation of the HDL subfractions demonstrated a marked difference in HDL_2 and only a minor variation in HDL_3 in both groups (**Fig. 1**).

The main HDL apoproteins apoA-I and A-II were both significantly higher, apoA-I, 1.6-fold and apoA-II, 1.3-fold, in hyperalphalipoproteinemia as compared to the low HDL group (Table 2). However, the apoA-I/A-II ratio was elevated only in women with high HDL-cholesterol; in men this ratio was not significantly increased in the high HDL subjects. This is in accordance with the higher apoA-II but lower apoA-I concentrations in male compared to female hyperalphalipoproteinemic subjects (Table 2). This suggests that elevation of apoA-II is also involved in male hyperalphalipoproteinemia whereas in female subjects hyperalphalipoproteinemia is due more to an elevation of apoA-I. The mean HDL cholesterol/serum apoA-I ratio was 0.26 in low HDL group and it was 0.42 in the high HDL group. This difference was apparent in both male and female subjects. Thus the HDL appears to be relatively enriched with cholesterol in all subjects with elevated HDL-cholesterol. In fact, the ratio of HDL-cholesterol/apoA-I appears to change in progression from low to high HDL as judged from relationships of HDL-cholesterol/apoA-I and HDL-cholesterol in both men and women (r = 0.92 in both sexes). A close relationship between the apoA-I and HDL_2 subfraction is suggested by their comparable cumulative distribution curves in the two groups (**Fig. 2**). Notably, the cumulative distribution of apoA-II resembled that of HDL_3. ApoA-I variants, which may underlie the exceptional HDL cholesterol concentrations, were not detected by isoelectric focusing method in either group.

The concentrations of apoE in the high-HDL and low-HDL groups are shown in Table 2. No differences could be detected in either the mean total concentration of apoE (Table 2) or in its cumulative distribution between the two groups (Fig. 2F). The phenotyping of apoE in both groups revealed phenotype distribution comparable to that of the Finnish population, except for one apoE3/1 subject in the low HDL group (**Table 3**). However, in all subjects the apoE phenotype influenced serum apoE con-

TABLE 2. Serum and lipoprotein lipids, apolipoproteins, and postheparin plasma lipoprotein lipase and hepatic lipase activities in hypo- and hyperalphalipoproteinemia

	Low HDL Cholesterol			High LDL Cholesterol			
	All (65)	Men (54)	Women (11)	All (62)	Men (13)	Women (49)	F[a]
Triglycerides, mg/dl	273 ± 45	273 ± 54	272 ± 89	94 ± 6	107 ± 54	90 ± 5	7.19^{NS}
VLDL-TG	195 ± 43	196 ± 52	191 ± 76	38 ± 6	55 ± 4	34 ± 4	5.84^{NS}
Cholesterol, mg/dl	245 ± 6	243 ± 7	249 ± 19	251 ± 5	236 ± 10	255 ± 6	1.21^{NS}
VLDL-C	47 ± 7	45 ± 7	56 ± 20	13 ± 1***	13 ± 2***	11 ± 1*	50.57
LDL-C	164 ± 5	166 ± 5	155 ± 13	151 ± 5	141 ± 10	155 ± 6	2.45^{NS}
HDL-C	34 ± 1	32 ± 1	38 ± 2[x]	87 ± 2***	82 ± 6***	89 ± 2***	291.7
HDL_2-C	15 ± 1	13 ± 0	19 ± 1[x]	62 ± 2***	57 ± 6***	64 ± 2***	253.1
HDL_3-C	19 ± 1	19 ± 1	18 ± 1	25 ± 1***	26 ± 2***	25 ± 1***	26.5
ApoA-I, mg/dl	128 ± 2.2	127 ± 2	132 ± 5	210 ± 2.8***	197 ± 8***	213 ± 3***,[x]	251.5
ApoA-II, mg/dl	43 ± 0.9	43 ± 1	43 ± 1	55 ± 1.1***	60 ± 3***	54 ± 1***,[x]	45.4
ApoE, mg/dl	4.9 ± 0.34	4.6 ± 0.3	6.3 ± 1.5	5.2 ± 0.3	4.6 ± 0.3	5.5 ± 0.4	2.8^{NS}
ApoA-I/A-II	3.03 ± 0.07	3.01 ± 0.08	3.12 ± 0.12	3.84 ± 0.08***	3.28 ± 0.12^{NS}	3.99 ± 0.08***,×××	44.5
HDL-C/apoA-I	0.26 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.42 ± 0.01***	0.42 ± 0.02***	0.42 ± 0.01***	171.1
HDL-C/apoA-II	0.78 ± 0.02	0.76 ± 0.02	0.88 ± 0.04[x]	1.63 ± 0.05***	1.37 ± 0.08***	1.70 ± 0.05***,×××	167.8
Lipoprotein lipase, μmol/h/ml	19.9 ± 0.82	20.1 ± 1.0	19.1 ± 1.5	27.8 ± 1.26***	25.2 ± 2.1*	28.5 ± 1.5***	15.03
Hepatic lipase, μmol/h/ml	38.5 ± 1.82	40.0 ± 2.1	30.9 ± 25[xx]	21.2 ± 1.47***	27.0 ± 5.0*	19.7 ± 1.3***	32.5

Numbers of subjects in each group are given in parenthesis. The results are mean ± SEM. The reference values of lipolytic enzymes are for normal women (n = 24): LPL, 27.2 ± 1.47 (range 14.4–43.3) μmol/h/ml; HL, 19.5 ± 1.14 (range 9.0–31.3 μmol/h/ml). The respective values for normal men were: LPL, 24.9 ± 1.0 (range 12.8–41.0) μmol/h/ml; HL, 31.9 ± 1.92 (range 11.2–70.3) μmol/h/ml (8).

*, $P < 0.05$; ***, $P < 0.001$ denotes significance of the difference between the resepctive high and low HDL groups (all vs. all, male vs. male, and female vs. female groups). [x], $P < 0.05$; [xx], $P < 0.01$; [xxx], $P < 0.001$ for difference between men versus women within the low-HDL or high-HDL groups.

[a]F denotes the variance ratio in one-way ANOVA of the low-HDL and high-HDL male and female groups (four groups). The significant value of $F_{0.05\ (3,123)} > 8.56$; NS, nonsignificant.

centrations (**Fig. 3**) which were higher in subjects with the apoE2 phenotype but lower in the apoE4 phenotype than in the apoE3/3 phenotype ($F = 16.34$, $P < 0.001$ by one-way analysis of variance). Serum apoE level did not correlate significant with serum triglyceride level ($r = 0.10$, NS) and neither did serum triglycerides vary significantly ($F = 1.93$, NS) according to the apoE phenotype (Fig. 3). The LDL cholesterol concentration was higher in subjects with the apoE4 phenotype and lower in subjects with the apoE2 phenotype in comparison with the E3/3 subjects ($F = 4.89$, $P < 0.01$), and a similar effect of the apoE phenotype could also be detected in serum apoB concentrations ($F = 4.88$, $P < 0.01$). However, neither serum apoA-I nor HDL cholesterol displayed any variation according to the apoE phenotype in the present study ($F = 1.85$, NS and 0.62, NS, respectively).

The activities of the two postheparin plasma lipolytic enzymes, LPL and HL, in men and women are also shown in Table 2. In all subjects with low HDL cholesterol the mean postheparin plasma HL activity was 82% higher, but the mean LPL activity was 28% lower than in the combined high HDL cholesterol group. In fact, the over-all variation of HL was significant by one-way analysis of variance whereas the LPL varied less significantly (Table 2). The cumulative distributions of the HL and LPL activities are shown in **Fig. 4**. This distribution of HL activity differed much more than that of LPL between the two groups (Fig. 4). Accordingly, the largest difference between the cumulative distributions of HL activity in the two groups was 51% ($P < 0.001$ by Kalmogorow-Smirnow's test) whereas that between the distributions of LPL activity was only 32% ($P < 0.01$).

Except for the HL and LPL activities, serum HDL_2 cholesterol also correlated with serum VLDL-triglyceride level and body mass index (**Table 4**). We therefore performed stepwise multiple regression analysis on the correlations of HDL_2 with body mass index, VLDL- triglycerides (expressed as logarithmic values), and the HL and LPL activities (Table 4), especially since the BMI had significant simple correlations also with log (VLDL-TG) ($r = 0.44$ $P < 0.001$), HL ($r = 0.32$, $P < 0.001$), and LPL ($r = -0.25$, $P < 0.01$). It appeared that all these variables contributed independently to the variance of HDL_2 cholesterol. Thus, 59% of the variance of HDL_2 cholesterol could be explained by BMI, VLDL-TG, and the lipase activities ($R^2 = 0.59$, $F = 44.0$, $P < 0.001$). The variation of age was also considerable in the present subjects (range between 19 and 64 yr). However, no significant correlations could be detected between age and serum lipoprotein concentrations in this study.

Serum apoA-I correlated significantly with both postheparin plasma HL and LPL activities ($r = -0.492$ and 0.484, respectively, $P < 0.001$) whereas the respective correlations of apoA-II with the lipase activities were much weaker, ($r = -0.179$, NS and $r = 0.287$, $P < 0.001$). The HDL-cholesterol/apoA-I ratio correlated significantly with both HL and LPL activities ($r = -0.501$ and 0.401, $P < 0.001$ for both). Notably, when standardized with serum HDL-

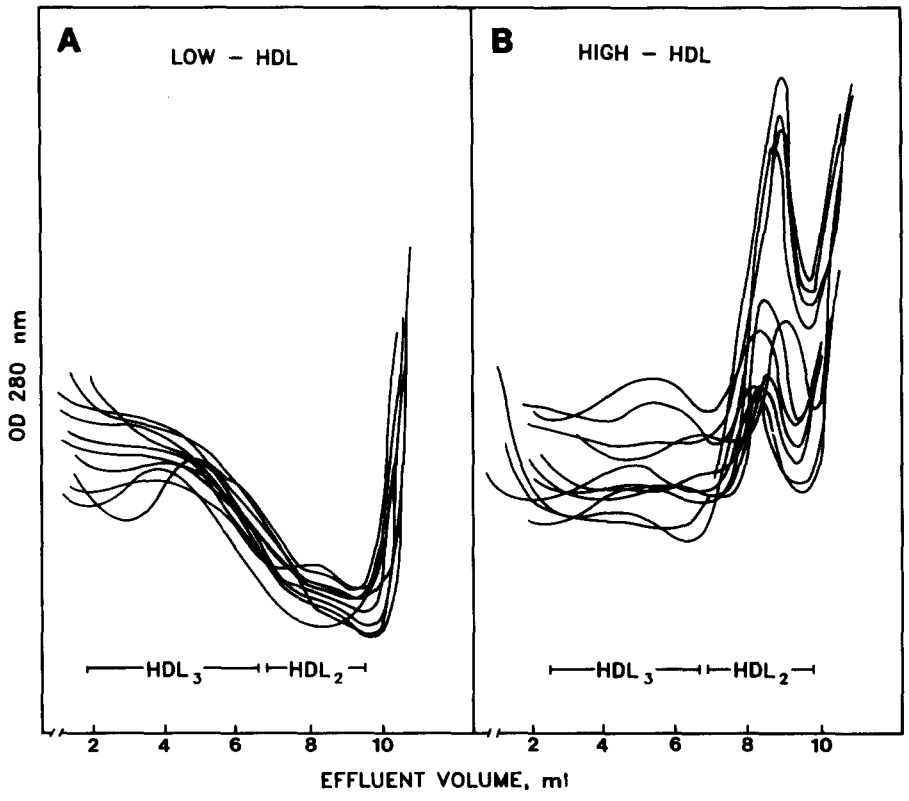

Fig. 1. HDL subfractions in hypo- and hyperalphalipoproteinemia. Rate-zonal separation of HDL subfractions was performed by the method of Groot et al. (26) in ten subjects with either low (A) or high (B) cholesterol concentration.

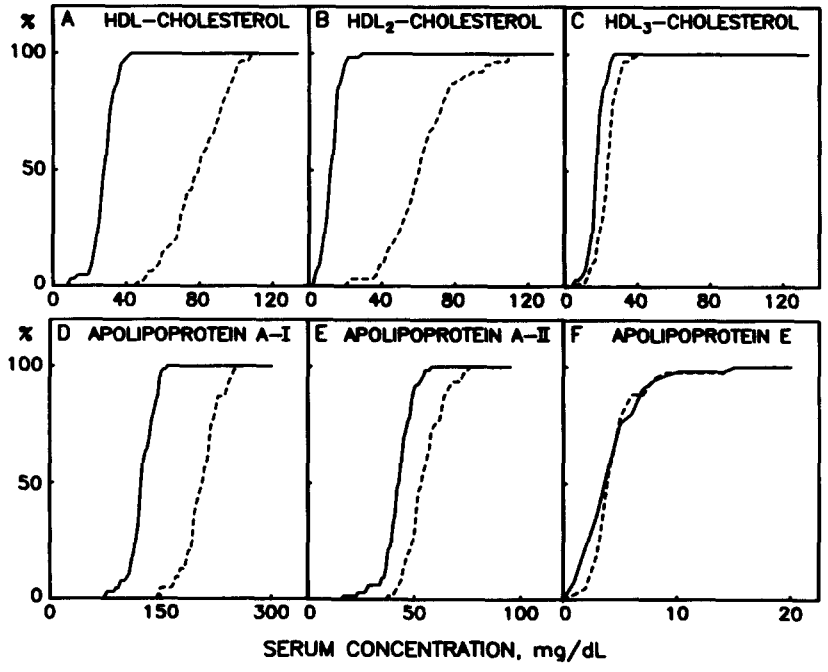

Fig. 2. Cumulative frequency distribution of HDL (A), HDL_2 (B), HDL_3 (C), apoA-I (D), apoA-II (E), and apoE (F) in the two groups with high (--) or low (—) HDL cholesterol concentration.

TABLE 3. Percentage distribution of different apoE phenotypes in the subjects with low or high HDL cholesterol concentrations

ApoE Phenotype	Low-HDL[a] %	(n)[c]	High-HDL[b] %	(n)	Finnish Population %
E4/4	10	(6)	4	(2)	5
E4/3	29	(18)	31	(16)	33
E4/2	0	(-)	2	(1)	1
E3/3	54	(33)	52	(27)	54
E3/2	5	(3)	9	(5)	6
E3/1	2	(1)	-	-	-
E2/2	0	(-)	2	(1)	0

[a]The observed phenotype distribution is not different from the expected (χ^2 = 5.48, $P > 0.10$).
[b]The observed phenotype distribution is not different from the expected (χ^2 = 5.53, $P > 0.10$).
[c]Number in parentheses is the number of subjects with the indicated apoE phenotype.

cholesterol, the above relationships of apoA-I with HL and LPL activities were no longer significant (r = −0.011 and 0.105, respectively). This suggests that the lipase activities might be more intimately involved in the regulation of HDL lipid than apoprotein content.

Of the 128 subjects studied, 12 men and 1 woman had previously suffered myocardial infarction (MI). The characteristics of this group are given in Table 1. One male MI subject had HDL cholesterol of 72 mg/dl, otherwise normal plasma lipoproteins but elevated blood pressure. The other MI subjects had very low HDL cholesterol (range 23–37.5 mg/dl). Only one subject had low HDL in the absence of other lipoprotein abnormalities whereas several low-HDL subjects with previous MI had coexistent hypertriglyceridemia (Table 1). The mean HL activity in the MI patients was 35.4 ± 2.6 μmol/h/ml (range 22.5–54.0 μmol/h/ml) and the mean LPL activity was 18.7 ± 2.1 μmol/h/ml (range 7.2–35.1 μmol/h/ml). Determination of apoE phenotypes revealed an increase in the E4 allele frequency (0.292) and a decrease in the E3 allele frequency (0.625) in this group in comparison with that in the Finnish population (0.227 and 0.733, respectively) (29). The subject with the apoE3/1 phenotype had HDL cholesterol of 22 mg/dl and had suffered a myocardial infarction 6 years prior to the study.

DISCUSSION

The two groups under study represent the extremes of HDL cholesterol distribution in a large random population sample. The variation of HDL level in populations is largely due to that of the HDL_2 subfraction (32) which was also the case in the present study. The profound differences in lipoprotein subclasses, especially the four-fold difference in the HDL_2 subfraction, allowed us to evaluate the impact of several factors on serum HDL at a population level. The current data demonstrate that both LPL and HL contribute significantly to the variation of HDL level and suggest that both enzymes are important in the etiology of hypo- and hyperalphalipoproteinemia. The relationship between serum HDL cholesterol concentration and postheparin plasma lipase activities has appeared thus far in only one random based study (23). Thus, the present data extend the knowledge on LPL and HL as determinants of HDL cholesterol in populations.

The two enzymes regulate serum HDL_2 in a reciprocal manner; high LPL elevates the HDL_2 but high HL is responsible for its degradation and consequently lower plasma HDL_2 level. The variation in serum HDL cholesterol concentration induced by LPL and HL becomes evident also under several physiological conditions and metabolic perturbations (9). Further, familial deficiencies of both lipolytic enzymes cause HDL abnormalities (33). Earlier studies on hyperalphalipoproteinemia have revealed an association between elevated LPL activity and high HDL (34). In addition, low or subnormal HL activity also leads to an elevation of the HDL level (18–20). In the present study both enzymes appeared to be responsible for HDL variation in subjects with both hypo- and hyperalphalipoproteinemia.

Other factors that influence serum HDL cholesterol concentration are HDL apoproteins and their metabolism. The absence of apoA-I variants in this study suggests that genetic variation of apoA-I studied by the isoelectric focusing method is not a common cause of extreme HDL concentrations, at least in the Finnish population. The two major apoproteins, apoA-I and A-II, varied according to the HDL cholesterol level. However, serum apoA-I displayed a wider range of variation than the apoA-II. Two

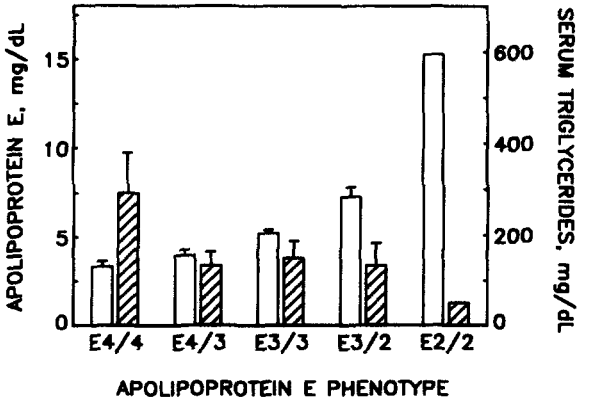

Fig. 3. Serum apolipoprotein E (open bar) and triglyceride (cross-hatched bar) concentrations according to the apolipoprotein E phenotype. Values are mean ± SEM except in the case of the single E2/2 phenotype subject. For statistical data see Results.

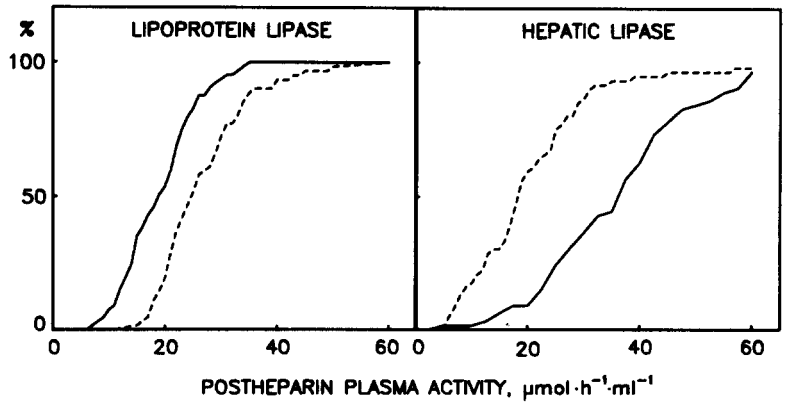

Fig. 4. Cumulative frequency distribution of lipoprotein lipase and hepatic lipase activities in the two groups with high (--) or low (—) HDL cholesterol concentration.

types of HDL particles are found within the 1.063–1.21 g/ml density interval, one containing apoA-I as the main protein, Lp(A-I), and another containing both apoproteins, Lp(A-I + A-II) (35, 36). The Lp(A-I) are mainly present in the HDL_2 subfraction whereas the Lp(A-I + A-II) circulates in the 1.125–1.21 g/ml density range. Substantial elevation of both apoA-I and HDL_2 cholesterol in hyperalphalipoproteinemia suggests that there are more HDL particles containing only apoA-I. Notably, the apoA-I/apoA-II ratio was significantly elevated in female high-HDL subjects in comparison to the female low-HDL subjects, indicating a more pronounced elevation of Lp(A-I) in women than in men (Table 2). In male hyper-HDL and hypo-HDL subjects, this ratio did not show any significant variation (Table 2). The reason for this difference of apoA-I/apoA-II ratio between two sexes is difficult to explain because of insufficient knowledge of the metabolism of Lp(A-I) and Lp(A-I + A-II). In men the HL but not LPL correlated significantly with the apoA-I/A-II ratio ($r = -0.463$, $P < 0.001$ and $r = -0.069$, NS, respectively) whereas in women, the apoA-I/apoA-II ratio correlated significantly with LPL ($r = 0.385$, $P < 0.01$) but not with HL ($r = -0.202$, NS). Therefore, one can speculate that HL and LPL have different roles in the metabolism of HDL particles with different apoprotein composition. This subject definitely requires further investigation.

The HDL density interval also contains lipoprotein particles with apoE as a major apoprotein. The reverse cholesterol transport has been attributed partly to these HDL particles (37) which circulate in lightest HDL_2 (or HDL_1) subfractions. Many subjects with abnormally high HDL_2 displayed marked accumulation of light HDL_2 particles (Fig. 1B). However, serum apoE concentration was similar in the two groups with high and low HDL levels (Fig. 2F). The distribution of apoE phenotypes was identical in the two population extremes and comparable to that observed in the Finnish population (Table 3). Serum HDL cholesterol and apoA-I concentrations were also similar in the different apoE phenotypes. These findings are in good accordance with earlier reports (29,38,39) on similar HDL levels in different apoE phenotypes. Apparently the metabolism of apoE within the HDL density range and its possible variation according to the apoE phenotype involves only a minor subpopulation of HDL particles which is not reflected in the total HDL level. Previous studies have documented variations of apoB and LDL cholesterol according to apoE phenotype in different populations (29,38,39). Similar variation of apoB was present in both hypo- and hyperalphalipoproteinemia. Thus, the presence of apoE4 and E2 seems to influence apoB metabolism irrespective of the HDL cholesterol concentration.

TABLE 4. Relationships of HDL_2 cholesterol concentration with body mass index (BMI), VLDL-triglycerides (VLDL-TG), and postheparin plasma LPL and HL activities according to simple (r) and multiple correlation (R) analyses

	All Subjects (127)		Low-HDL (65)		High-HDL (62)		Men (67)		Women (60)	
	r	R	r	R	r	R	r	R	r	R
BMI	−0.521***	−0.235**	−0.033	−0.007	−0.241	−0.015	−0.546***	−0.464***	−0.366**	+0.001
Log VLDL-TG	−0.625***	−0.301***	−0.229	−0.203	−0.088	+0.081	−0.377**	−0.137	−0.583***	−0.419***
LPL activity	+0.510***	+0.359***	−0.033	−0.016	+0.399***	+0.395***	+0.239*	+0.175	+0.565***	+0.465***
HL activity	−0.570***	−0.456***	−0.458***	−0.442***	−0.201	−0.200	−0.421***	−0.377**	−0.355**	−0.182

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Several subjects with hypoalphalipoproteinemia had a concomitant elevation of serum triglycerides (type IIB or IV hyperlipoproteinemia). It was, therefore, interesting to note that postheparin plasma LPL and HL activities did not differ significantly between hypoalphalipoproteinemic subjects with or without hypertriglyceridemia (data not shown). Thus, low HDL appears primarily to coincide with altered lipolytic enzyme activities whereas this lipoprotein abnormality is heterogenous with respect to the triglyceride concentration. Further studies are needed to elucidate the interactions between HDL and triglyceride metabolism in hypoalphalipoproteinemia.

Thirteen subjects in the present study had a previous myocardial infarction. The marked (12-fold) difference in the previous prevalence of MI between the low and high HDL groups is striking but not unexpected. Ten MI patients with low-HDL cholesterol had elevated serum triglycerides (type IIB or V hyperlipoproteinemia), one had hypercholesterolemia, whereas only one low-HDL subject with previous MI had otherwise normal serum lipoproteins (Table 1). Thus, 1 of 17 low-HDL subjects with normal lipoprotein phenotype had a previous MI whereas 10 of 39 low-HDL subjects with type IIB or IV hyperlipoproteinemia had had an MI (Table 1). Accordingly, the presence of hypertriglyceridemia appears to increase the risk of MI by 4-fold in hypoalphalipoproteinemia. This suggests that low-HDL in MI patients probably is more likely a marker of abnormal metabolism of triglyceride-rich lipoproteins (and/or their remnants?) than an independent risk factor. The small number of MI patients in the present study may challenge this proposal. However, the data-basis used for selection of the low-HDL group in the present study involved about 5,000 randomly selected Finns. Accordingly, the low extreme of HDL cholesterol in this random population sample should well represent those subjects at high risk of MI with respect to reduced HDL cholesterol.

The increase of apoE4 phenotypes in the MI subjects agrees with earlier reports on an increased prevalence of the apoE4 allele in coronary heart disease (CHD) (39,40). Moreover, the E4 allele frequency of 0.292 is very close to the E4 allele frequency of 0.324 observed in coronary angiography-positive Finns (40). Therefore, the apoE4 phenotype may be an additional factor that increases the risk for CHD independently of low HDL cholesterol. The concomitant variation of apoB according to the apoE phenotype suggests that disturbed LDL metabolism at the hepatic apoB/E receptor sites probably mediates the influence of apoE4 on CHD risk.

Deficient or abnormally low HL and LPL activity have been suggested to be risk factors for CHD (41,42). In this study none of the 13 subjects with previous MI had abnormally low HL activity. Low LPL activity (7.2 μmol/h/ml) was evident in one subject with MI but the mean LPL activity of the MI subjects was similar to that of the other subjects with low HDL. This suggests that low LPL activity might not promote the CHD risk if other risk factors such as low HDL cholesterol or elevation of serum triglycerides are not present. Likewise, lipolytic enzyme activities have been reported to be similar in patients with myocardial infarction and in control subjects (43).

In summary, the present results demonstrate a role for both LPL and HL and for factors that regulate the concentrations of apoA-I and A-II as determinants of serum HDL (HDL_2) cholesterol, but rule out a major role of both apoA-I and apoE variants in hypo- and hyperalphalipoproteinemia.

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