High density lipoproteins with differing apolipoproteins: relationships to postprandial lipemia, cholesteryl ester transfer protein, and activities of lipoprotein lipase, hepatic lipase, and lecithin:cholesterol acyltransferase

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Abstract To gain insight into metabolic determinants of high density lipoproteins (HDL) containing apolipoproteins A-I and A-II (LpA-I/A-II) and those containing A-I, but devoid of A-II (LpA-I), the plasma concentration of LpA-I and LpA-I/A-II within the HDL_2 and HDL_3 density spectrum was measured in 14 normolipidemic male subjects on a standardized diet. Apolipoprotein plasma concentrations of HDL subspecies were compared with the magnitude of postprandial lipemia, activities of lipoprotein lipase and hepatic lipase in postheparin plasma, plasma lecithin:cholesterol acyltransferase (LCAT) activity, and cholesteryl ester transfer protein (CETP) mass. Plasma levels of LpA-I/A-II were 2.5 times higher than levels of LpA-I (123 ± 20 vs. 48.3 ± 22.1 mg protein/dl) and the partition of LpA-I and LpA-I/A-II between HDL_2 and HDL_3 differed in that the proportion of LpA-I associated with HDL_2 was greater than that of LpA-I/A-II (23 ± 19 vs. 6 ± 6%, $P < 0.002$). With increasing levels of HDL_2, the proportion of LpA-I in HDL_2 increased ($P < 0.002$). Furthermore, levels of LpA-I and LpA-I/A-II were strongly correlated within the HDL_2, but not within the HDL_3 density region. Plasma levels of LpA-I, but not LpA-I/A-II, were inversely correlated with the magnitude of postprandial lipemia. However, activities of lipoprotein lipase and hepatic lipase tended to show stronger associations with the partition of LpA-I/A-II between HDL_2 and HDL_3 than with that of LpA-I. Within the HDL_3, but not the HDL_2 density spectrum, LpA-I/A-II exhibited a positive association with plasma LCAT activity, while LpA-I displayed an inverse association with plasma CETP mass. These results are consistent with differences in substrate properties of LpA-I and LpA-I/A-II for lipoprotein-modifying enzymes and imply different, but overlapping metabolic pathways of LpA-I and LpA-I/A-II.—**Mowri, H-O., J. R. Patsch, A. Ritsch, B. Föger, S. Brown, and W. Patsch.** High density lipoproteins with differing apolipoproteins: relationships to postprandial lipemia, cholesteryl ester transfer protein, and activities of lipoprotein lipase, hepatic lipase, and lecithin:cholesterol acyltransferase. *J. Lipid Res.* 1994. **35:** 291–300.

Supplementary key words HDL subfractions • lipolytic enzymes • triglyceride-rich lipoproteins

An inverse relation between high density lipoprotein (HDL) cholesterol and incidence and/or prevalence of coronary heart disease (CHD) has been firmly established in numerous epidemiologic studies (1, 2), but the mechanism(s) whereby HDL provide protection from CHD are not fully understood. Traditionally, human HDL are divided into two major subclasses termed HDL_2 and HDL_3 (3). These HDL subclasses are heterogeneous with respect to physical, chemical, and functional properties (4). The proportions of the two major HDL apolipoproteins A-I (apoA-I) and A-II (apoA-II) differ among particles within the HDL_3 density spectrum. Furthermore, molar ratios of apoA-I to apoA-II in some HDL_2 fractions are too high to allow the presence of one molecule of apoA-II on each particle (5). Indeed, apoA-I-HDL that contain apoA-II (LpA-I/A-II) or are devoid of apoA-II (LpA-I) can be isolated by immunochromatography (6).

A number of studies indicate that LpA-I and LpA-I/A-II are metabolically distinct and may perform different functions. ApoA-I injected as part of LpA-I particles is catabolized at a higher rate than apoA-I injected as part of LpA-I/A-II particles (7). Particles containing only apoA-I may be the physiologic acceptor of cellular cholesterol (8), and HDL_2 containing both apoA-I and apoA-II constitute a better substrate for hepatic lipase

Abbreviations: HDL, high density lipoproteins; LpA-I, HDL containing apoA-I; LpA-I/A-II, HDL containing apoA-I and apoA-II; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; TGRL, triglyceride-rich lipoproteins; LPL, lipoprotein lipase; HL, hepatic lipase.

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than HDL_2 containing apoA-I only (9). There is evidence to suggest that plasma levels of LpA-I reflect the protective function of HDL better than plasma levels of LpA-I/A-II. The well-established male–female difference in plasma levels of HDL_2 was shown to result from increased concentrations of LpA-I in the HDL_2 fraction of females (10), and the presence of CHD was inversely correlated with levels of LpA-I, but not LpA-I/A-II, in a study comparing patients with angiographically verified CHD and controls (11). Furthermore, transgenic mice overexpressing human apoA-I exhibit enhanced protection against diet-induced atherosclerosis when compared with mice overexpressing both human apoA-I and apoA-II (12).

HDL are strongly affected by the metabolism of triglyceride-rich lipoproteins (TGRL). Catabolism of TGRL is associated with transfer of surface components from TGRL to HDL, leads to the anabolic formation of HDL_2 (13), and is correlated with activity of lipoprotein lipase (LPL), plasma levels of HDL cholesterol and HDL_2 (14, 15). Conversely, elevated levels of TGRL resulting from defective clearance or from partial saturation of the clearance pathway by hepatic TGRL lead to redistribution of triglyceride into HDL at the expense of cholesteryl ester via cholesteryl ester transfer protein (CETP). Hepatic lipase (HL) may then convert the triglyceride-enriched HDL_2 into smaller, denser HDL_3 particles (16).

In contrast to the relationship between HDL cholesterol, HDL_2 and HDL_3 on the one hand and TGRL on the other, that between LpA-I and LpA-I/A-II and TGRL is poorly understood. In a previous study, the molar ratio of apoA-I to apoA-II in HDL_2 was correlated with lipoprotein lipase activity and HDL_2 levels, and was inversely associated with hepatic lipase activity and the magnitude of postprandial lipemia (15). As differences in the molar apoA-I/apoA-II ratios of HDL_2 may have resulted from different proportions of LpA-I and LpA-I/A-II among individuals, we reasoned that the metabolism of TGRL plays a role in plasma levels of LpA-I and LpA-I/A-II and that selective interactions of LpA-I and LpA-I/A-II with lipoprotein-modifying enzymes account, at least in part, for their uneven distribution among HDL_2 and HDL_3. To test these hypotheses, we quantified LpA-I and LpA-I/A-II within the density spectrum of HDL_2 and HDL_3 and determined their relationship to the magnitude of postprandial lipemia, activities of LPL, HL, and LCAT, and CETP mass. We report here that the plasma levels of LpA-I-protein, but not of LpA-I/A-II-protein, are inversely related to the magnitude of postprandial lipemia. Activities of hepatic and lipoprotein lipase are associated with the distribution of LpA-I/A-II and, to a lesser extent, LpA-I into HDL_2 and HDL_3. However, LCAT activity is strongly correlated with LpA-I/A-II in HDL_3, while LpA-I in HDL_3 shows a strong inverse correlation with CETP mass. These differences in associations among HDL subfractions and activities of lipoprotein-modifying enzymes indicate distinct metabolic pathways for LpA-I and LpA-I/A-II.

METHODS

Study subjects

This study was approved by the Human Subject Review Committee of Baylor College of Medicine, and informed consent was obtained from participants. Volunteers were recruited by announcement at the institution campus and participation in the study was remunerated. Fourteen subjects were enlisted in the study. Inclusion criteria were: healthy males between the ages of 20–50 years; within 30% of ideal body weight as determined by the Metropolitan Life Insurance tables (17); and less than 240 mg/dl for fasting plasma cholesterol, 200 mg/dl for fasting plasma triglycerides, and 175 mg/dl for LDL-cholesterol through initial screening procedures. None of the subjects had a history or signs of liver, heart, kidney, or thyroid disease. For 2 weeks prior to metabolic studies, subjects ate a diet containing 45% calories from carbohydrate, 15% from protein, and 40% from fat for 14 days prior to metabolic studies. Dietary cholesterol was restricted to 300 mg per day. Eleven of the 14 participants consumed food prepared by the Metabolic Kitchen of The Methodist Hospital. In these subjects, levels of dietary components were determined from an analysis on all foods for 1 week using the Nutrition Data System software, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (Food Database version 4A; Nutrient Database version 19). Mean calculated intake of carbohydrates, protein, and fat amounted to 40, 16, and 44% of the caloric intake. Mean daily cholesterol intake was 279 mg/dl, and the percent of total fatty acids was 17, 7, 44, and 15% for 16:0, 18:0, 18:1, and 18:2 fatty acids. Three subjects consumed diets with similar dietary goals on an outpatient basis. The caloric distribution among dietary food components as determined from 4-day food records was similar to that listed above.

Test meal

At the end of the dietary period, a standard liquid fat meal, whose composition and preparation have been described previously (18), was administered to fasting subjects. Each individual ingested 65 g fat/m^2 surface. Blood samples were collected prior to and 2, 4, 6, and 8 h after ingestion of the fatty meal. The magnitude of postprandial lipemia was quantified as the area under the time-dependent triglyceride-level curve. This area was defined by two lines, i.e., one connecting the individual triglyceride values and one originating at the 0-hr triglyceride level parallel to the abscissa. This area (mg/dl

of plasma times 8-h TG area) was calculated by the trapezoidal rule as described (18). The reproducibility of this fat tolerance test within individuals has been demonstrated previously (18, 19).

Laboratory methods

Blood was collected into tubes containing 1.5 mg EDTA/ml of blood, and plasma was separated by centrifugation (1500 rpm, 20 min, 4°C). Cholesterol and triglyceride in plasma were measured by enzymatic procedures (20, 21). HDL cholesterol was determined by measuring cholesterol in the supernatant liquid after precipitation of the plasma with $MgCl_2$ and dextran sulfate (22). LDL cholesterol was calculated according to Friedewald, Levy, and Fredrickson (23). ApoA-I and apoB levels were measured by RIA methods (24, 25). ApoA-II was measured by radial immunodiffusion (Immuno AG, Vienna, Austria). The coefficients of variation (including interassay and intraassay variability) measured using aliquots of internal quality control pools were 2.5% for cholesterol, 2.7% for triglyceride, 5.2% for LDL cholesterol, 3.7% for HDL cholesterol, and ranged from 8 to 9% for apoA-I, apoB, and apoA-II.

For isolation and quantification of HDL subfractions, plasma was subjected to zonal ultracentrifugation (26). Rotor fractions under the HDL_2 and HDL_3 peaks were pooled, dialyzed against EDTA-saline, and analyzed for protein content (27).

For separation of A-I- and A-I/A-II-subfractions of HDL_2 and HDL_3, immunoaffinity chromatography was used as described previously (9). Briefly, Mab 32, a monoclonal anti-apoA-II antibody (28), was purified from murine ascites fluid by immunoaffinity chromatography using HDL_3 immobilized to Sepharose 4B. Affinity-purified Mab 32 was covalently coupled to CNBr-activated Sepharose 4B at a ratio of 9–11 mg per 1 g of gel. The Mab 32–Sepharose 4B was extensively washed with several cycles of 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.0, and 0.1 M Na acetate, 0.5 M NaCl, pH 4.0, followed by 0.2 M glycine, pH 2.8. The gel was then equilibrated in 0.05 M Tris-HCl, pH 7.4, containing 0.02% NaN_3 and stored at 4°C. HDL_2 or HDL_3 was diluted to a concentration of 0.5 mg protein/ml with 10 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl, 1 mM EDTA, and 0.02% NaN_3, and applied to Mab–Sepharose columns (1 × 7 cm) and eluted at a flow rate of 8 ml/h. The eluates were recycled twice through the columns prior to collecting unbound HDL. Bound HDL was eluted with 0.2 M glycine buffer, pH 2.8, and collected into tubes containing 1 M Tris-HCl, pH 8.0. The capacity of the gel to retain apoA-II-containing particles determined by monitoring apoA-II in the unbound fraction by electrophoretic procedures (see below) was greater than 0.9 and 0.55 mg protein per ml gel for HDL_2 and HDL_3, respectively.

Fractions were pooled, dialyzed against 0.15 M NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, and analyzed for chemical composition. Lipid phosphorus was determined by the procedure of Bartlett (29), and protein was quantified by the method of Lowry et al. (27) using bovine serum albumin as standard. Triglyceride, cholesterol, and cholesteryl ester were determined by enzymatic procedures (20, 21, 30). After immunoaffinity chromatography of five HDL_2 preparations, mean recoveries (SD) of protein, phospholipids, triglyceride, and cholesterol were 90.2 (5.1), 89.4 (6.1), 93.3 (11.4), and 83.8 (4.0) percent, respectively, and did not differ from each other (analysis of variance). For five HDL_3 preparations, mean (SD) recoveries of protein, phospholipid, triglyceride, and cholesterol were 84.7 (5.1), 88.7 (7.3), 92.1 (7.4), and 86.0 (7.6), respectively. Again, no statistically significant difference in recoveries of HDL_3 constituents was observed. The reproducibility of separating particles with or without apoA-II was determined in three aliquots of each of two HDL_2 and two HDL_3 preparations. The mean (SD) percentage distribution of LpA-I and LpA-I/A-II was 65.5 (0.7) and 34.5 (0.7) percent in one HDL_2 preparation, and 44.3 (2.3) and 55.7 (2.3) percent in the other. The mean (SD) percentage distribution of LpA-I and LpA-I/A-II was 28.3 (1.8) and 71.7 (1.8) percent in one HDL_3 preparation, and 26.7 (0.3) and 73.3 (0.3) percent in the other. Plasma levels of LpA-I and LpA-I/A-II in HDL_2 and HDL_3 are expressed as mg/dl lipoprotein-protein and were corrected for recovery of lipoprotein-protein that occurred after zonal isolation of HDL_2 and HDL_3.

For estimation of particle size, HDL fractions were subjected to electrophoresis in 4–30% polyacrylamide gels (31). Apolipoproteins of HDL fractions were separated by polyacrylamide gel electrophoresis in 0.1% SDS (32) or by isoelectric focusing (33). Relative abundance of apoA-I and apoA-II was determined by scanning of isoelectric focusing gels using a laser densitometer (LKB Ultrascan XL, Pharmacia LKB Biotechnology Inc., Piscataway, NJ) as described (34).

To release LPL and HL into the circulation, heparin sodium from beef lung (Upjohn C., Kalamazoo, MI) was injected intravenously at a dose of 2,280 U/m^2. Postheparin blood was collected 15 min after injection of heparin. Plasma was separated immediately by centrifugation at 4°C and stored at −70°C. Sonicated emulsions of [9,10-^{3}H]oleic acid-labeled trioleoylglycerol (Amersham, Arlington Heights, IL) in phosphatidylcholine and gum arabic were employed as substrates for estimation of LPL and HL activity, respectively (35, 36). To LPL assay mixtures, heat-inactivated fasted rat serum was added as a source of apoC-II; HL was inhibited by goat anti-human HL IgG. For assay of HL, LPL was suppressed by raising the NaCl concentration of the incubation mixture to 1 M and omitting the source of apoC-II. Lipase activities were

TABLE 1. Lipid, lipoprotein-lipid, and apolipoprotein plasma levels of study subjects

	Chol	TG	LDL-C	HDL-C	ApoB	ApoA-I	ApoA-II
				mg/dl			
Mean	176	78	112	49	80	140	31
1 SD	35	27	36	12	29	19	6
Range	117–239	46–128	55–177	33–74	48–145	122–175	25–45

measured by incubating for 30 min at 25°C and at a pH of 8.5 (35, 36). Activity is expressed in milliunits (mU) which correspond to 1 nmol of fatty acids released per min.

CETP mass was quantified using an immunoradiometric assay (IRMA) with polyclonal anti-CETP antibody as described (37). Briefly, polystyrol tubes (12 × 75 mm, MaxiSorp^{R} Star Tubes, Nunc, Roskilde, Denmark) were coated with 250 μl affinity-purified anti-CETP antibody solution containing 10 μg/ml in 0.01 M bicarbonate buffer, pH 9.6, at room temperature for 2 h. After rinsing two times with PBS, 200 μl of sample in PBS, supplemented with 2% BSA and 1% Triton X-100, was added to each tube. After an incubation of 30 min at room temperature and two washes with PBS, 200 μl of ^{125}I-labeled antibody solution (100,000 cpm) was added and incubated for 4 h. After three rinses with PBS, the radioactivity bound to each tube was measured in a gamma-counter (1272 Clinigamma, Pharmacia-LKB, Uppsala, Sweden).

Activity of LCAT in plasma was measured using an artificial substrate consisting of apoA-I, 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), and [1α, 2α(n)-^{3}H]cholesterol (40–60 Ci/mmol, Amersham, Arlington Heights, IL). Lipid-protein complexes with a molar composition of POPC–cholesterol–apoA-I of 100:2:1 were prepared by the cholate dialysis procedure (38). Aliquots of plasma (0.2 μl) were incubated with reconstituted particles (600 pmol cholesterol) in 100 μl of 50 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl, 1 mM EDTA, 2 mM dithiothreitol, and 0.25% human serum albumin for 1 h at 37°C. The incubation was stopped by extraction of lipids with 0.5 ml methanol and 1.0 ml hexane. Aliquots of the hexane layer were applied to Silica G-60 minicolumns (0.5 × 3 cm), and cholesteryl esters were eluted twice with 0.75 ml of hexane–diethylether 6:1 (v/v). The eluate was drained into vials containing Scintiverse E for counting of radioactivity (38).

For comparison of groups and estimation of associations, nonparametric tests were used to avoid normalizing transformations such as logarithms and square-roots because of their lack of intuitive appeal and resulting loss of the original scale.

RESULTS

Plasma lipid, lipoprotein lipid, and apolipoprotein levels from the 14 study subjects are shown in **Table 1.** LpA-I/A-II and LpA-I were isolated from HDL_2 and HDL_3 obtained by zonal ultracentrifugation of fasting plasma. As illustrated in **Figs. 1A and 1B,** the unbound fraction of HDL_2 and HDL_3 was devoid of apoA-II. Dye uptake ratios of apoA-I/apoA-II in isoelectric focusing gels were 6.4 ± 1.4 and 4.9 ± 1.0 in A-I/A-II-HDL_2 and A-I/A-II-HDL_3, respectively (mean ± SD, n = 9, $P < 0.05$, Mann-Whitney test). As estimated by nondenaturing gra-

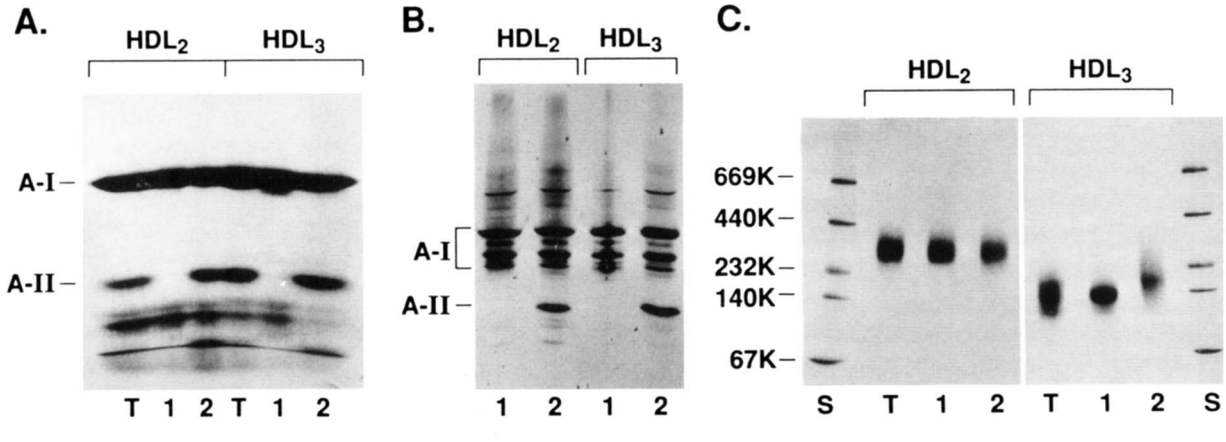

Fig. 1. Analysis of postabsorptive HDL subpopulations by SDS-polyacrylamide gel electrophoresis (A), isoelectric focusing (B), and 4–30% polyacrylamide gel electrophoresis under nondenaturing conditions (C); lanes T, 1, and 2 are total, A-I-, and A-I/A-II-HDL_2 or -HDL_3, respectively. Molecular weight standards are shown in lanes S.

TABLE 2. Weight percentage chemical composition of HDL subfractions

Subfraction	Protein	Phospholipids	Triglyceride	Cholesterol	Cholesteryl Ester
			wt %		
A-I-HDL_2	37.0 ± 1.9[a]	34.7 ± 2.7[b]	4.3 ± 1.7	5.5 ± 1.0[c]	18.5 ± 1.1[d]
A-I/A-II-HDL_2	44.6 ± 2.3[a]	31.7 ± 2.8[b]	2.9 ± 1.1	3.9 ± 0.8[c]	17.0 ± 1.5[d]
A-I-HDL_3	57.1 ± 4.0	21.6 ± 4.4	2.7 ± 0.7	2.5 ± 0.8	16.1 ± 2.5
A-I/A-II-HDL_3	56.0 ± 3.4	23.2 ± 3.6	2.4 ± 0.9	2.0 ± 0.4	16.4 ± 1.0

Results are means ± SD of eight different subjects.
[a,b,c,d] $P < 0.05$ from each other.

dient gel electrophoresis, sizes of HDL_2 with and without apoA-II were 11.0 ± 0.1 and 10.9 ± 0.1 nm, respectively. Sizes of A-I- and A-I/A-II-HDL_3 were 9.3 ± 0.1 and 9.6 ± 0.2 nm, respectively (Fig. 1C). When compared to A-I-HDL_2, A-I/A-II-HDL_2 exhibited a higher content of protein, but a lower content of phospholipids, free cholesterol, and cholesteryl ester. In contrast, HDL_3 subspecies did not show this difference with respect to chemical composition (**Table 2**).

Plasma protein concentrations of HDL and various HDL subfractions are displayed in **Table 3.** Levels of LpA-I and LpA-I/A-II in the entire HDL spectrum were 48 ± 22 mg/dl and 123 ± 20 mg/dl, respectively, in our 14 study subjects ($P < 0.001$, Mann-Whitney test). The interindividual variability of LpA-I protein was larger (range 25.0–102.0 mg/dl) than that of LpA-I/A-II protein (range 86.3–153.8 mg/dl). An even higher degree of variation among subjects was found with the HDL_2 subclass and, here in particular, for A-I-HDL_2 (range 1.4–49.1 mg/dl). In contrast, levels of HDL_3, A-I-HDL_3, and A-I/A-II-HDL_3 were fairly similar among subjects. Furthermore, the distribution of LpA-I and LpA-I/A-II between HDL_2 and HDL_3 differed in that the proportion of LpA-I associated with HDL_2 was greater than that of LpA-I/A-II (23 ± 19% vs. 6 ± 6%, $P = 0.0015$).

Plasma levels of LpA-I within the entire HDL spectrum were correlated with levels of A-I-HDL_2, A-I/A-II-HDL_2, and A-I-HDL_3 (**Table 4**). Levels of LpA-I/A-II showed strong associations with levels of A-I/A-II-HDL_3, but not with A-I/A-II-HDL_2. While both A-I- and A-I/A-II-HDL_2 strongly correlated with HDL_2 and with each other, the proportion of HDL_2 made up by A-I-HDL_2 increased with increasing HDL_2 levels (Spearman R = 0.7626, $P = 0.0015$). Neither A-I- nor A-I/A-II-HDL_2 correlated with the respective fraction within HDL_3. In contrast to their association within the HDL_2 density spectrum, levels of A-I/A-II particles were not correlated with levels of particles devoid of apoA-II within the HDL_3 density range.

Activities of lipoprotein lipase and hepatic lipase in postheparin plasma as well as plasma LCAT activity and CETP plasma concentrations in our study subjects are displayed in **Table 5.** In agreement with our previous studies (15), there was a significant inverse association of the magnitude of postprandial lipemia with LPL activity (Spearman R = −0.6993, $P = 0.011$) and with HDL_2 levels (R = −0.6967, $P = 0.006$). No relation was observed between lipemia and HL activity (R = 0.4685, $P = 0.12$), LCAT activity (R = −0.148, $P = 0.63$), or with CETP mass (R = 0.1868, $P = 0.58$). The correlations between the magnitude of postprandial lipemia and plasma levels of the various HDL subfractions are illustrated in **Fig. 2.** Inverse associations were noted between lipemia and LpA-I, and even stronger inverse relationships existed between lipemia and A-I- and A-I/A-II-HDL_2, but no associations were found between lipemia and Lp-A-I/A-II, A-I- or A-I/A-II-HDL_3. When A-I- and A-I/A-II-HDL_2 were expressed as percentage of total HDL_2, a borderline significant inverse association was found only between A-I-HDL_2 and lipemia (R = −0.4857, $P = 0.07$). This is consistent with our previous findings showing an inverse relationship between lipemia and molar ratios of apoA-I/apoA-II in HDL_2 (15).

Table 6 compares levels of HDL subspecies with activities of lipoprotein lipase, hepatic lipase, and LCAT, and CETP mass. Plasma levels of the entire HDL_2 fraction, A-I-HDL_2, and A-I/A-II-HDL_2 were inversely related to HL activity, but only HDL_2 and A-I/A-II-HDL_2 correlated with LPL activity. A-I-HDL_3 showed a strong inverse association with CETP mass, while A-I/A-II-HDL_3 exhibited a strong correlation with LCAT activity. The distribution of LpA-I/A-II between HDL_2 and HDL_3 was

TABLE 3. Plasma concentration of HDL and HDL subfractions

HDL Subfraction	Mean	SD	Range
		mg/dl	
HDL protein	171.3	30.1	123.3–232.5
LpA-I protein	48.3	22.1	25.0–102.0
LpA-I/A-II protein	123.0	20.0	86.3–153.8
HDL_2 protein	21.0	21.9	4.4–71.5
A-I-HDL_2 protein	13.9	15.7	1.4–49.1
A-I/A-II-HDL_2 protein	7.2	6.3	2.1–22.3
HDL_3 protein	150.3	25.1	95.2–184.7
A-I-HDL_3 protein	34.4	10.6	22.6–52.9
A-I/A-II-HDL_3 protein	115.8	21.5	70.6–150.2

Results are from 14 subjects and are expressed in mg/dl.

TABLE 4. Spearman correlation matrix of various HDL subfraction protein concentrations in plasma

	LpA-I	LpA-I/A-II	A-I-HDL_2	A-I/A-II-HDL_2	A-I-HDL_3	A-I/A-II-HDL_3
LpA-I	1.000	−0.007	0.833[b]	0.578[a]	0.574[a]	−0.301
LpA-I/A-II	-	1.000	−0.218	−0.090	0.235	0.903[b]
A-I-HDL_2	-	-	1.000	0.829[b]	0.152	−0.539[a]
A-I/A-II-HDL_2	-	-	-	1.000	0.174	−0.402
A-I-HDL_3	-	-	-	-	1.000	0.099
A-I/A-II-HDL_3	-	-	-	-	-	1.000

[a] $P < 0.05$.
[b] $P < 0.001$.

correlated with lipoprotein lipase activity, and exhibited an inverse association with hepatic lipase activity. The ratio of A-I-HDL_2 to A-I-HDL_3 exhibited only an inverse association with hepatic lipase activity, and the partition of LpA-I and LpA-I/A-II between HDL density fractions showed no relationship with LCAT activity or CETP mass.

DISCUSSION

This study confirms and extends previous observations (7, 8, 9, 12) suggesting that LpA-I and LpA-I/A-II are functionally diverse HDL populations. The main new findings relate to distinct relationships of density subfractions of LpA-I and LpA-I/A-II with mass or activities of lipoprotein-modifying enzymes. Among the differences observed, the correlation of A-I/A-II-HDL_3 levels with LCAT activity and the inverse association of A-I-HDL_3 with CETP mass were most notable. In addition, plasma levels of LpA-I were inversely correlated with the magnitude of postprandial lipemia while plasma levels of LpA-I/A-II were not. Furthermore, activities of LPL and HL tended to show stronger associations with the partition of LpA-I/A-II between HDL_2 and HDL_3 than with that of LpA-I. Additional associations may exist among other variables, but may not have been detected in this study due to lack of statistical power.

Levels of LpA-I and LpA-I/A-II in our study subjects (Table 3) were similar to those of another population measured with a different methodology (39). Consistent with data from Luc et al. (39), LpA-I showed a larger intraindividual variability than LpA-I/A-II, and A-I-HDL_2 exhibited the highest variability between subjects. The majority of LpA-I/A-II was found within the HDL_3 density spectrum, and the fraction of LpA-I present in HDL_2 was much larger than that of LpA-I/A-II as described previously. Furthermore, HDL_2 contained more LpA-I than LpA-I/A-II as reported previously by some investigators (40), but not others (10). With higher HDL_2 levels, the proportion of LpA-I in HDL_2 increased as suspected previously (15). Thus, LpA-I appears to be the major contributor to the overall HDL_2 concentration in plasma while LpA-I/A-II seems to hold this role for HDL_3.

The chemical composition of LpA-I and LpA-I/A-II differed in the HDL_2 density range in that A-I-HDL_2 contained less protein, but more phospholipid, cholesterol, and cholesteryl ester than A-I/A-II-HDL_2. No compositional differences of LpA-I and Lp-A-I/A-II were found in the HDL_3 flotation range (Table 2). Upon gradient gel electrophoresis, one major population was found within each of the four subfractions isolated. Trace quantities of smaller particles containing apoA-I, but not apoA-II were found in some subjects. Such small-sized A-I-HDL_3 has also been found by other investigators in small quantities (8, 41). Because of its high protein content and small size, the flotation properties of this population may not have allowed complete separation from residual plasma proteins under the ultracentrifugal conditions applied. Hence, the

TABLE 5. Activities of lipoprotein lipase, hepatic lipase, and LCAT, and CETP mass in study subjects

	Lipoprotein Lipase Activity	Hepatic Lipase Activity	LCAT Activity	CETP Mass
	mU		*nmol/ml plasma/h*	*μg/ml*
N	12	12	13	11
Mean	372.5	335.6	112.9	1.18
1 SD	99.2	185	38.0	0.21
Range	210–526	78–703	59.3–185.9	0.94–1.65

Activities of lipoprotein lipase and hepatic lipase are expressed in milliunits which correspond to 1 nmol of fatty acids released per min per ml of plasma. LCAT activity is expressed in nmol/ml plasma per h. CETP mass is expressed in μg/ml of plasma.

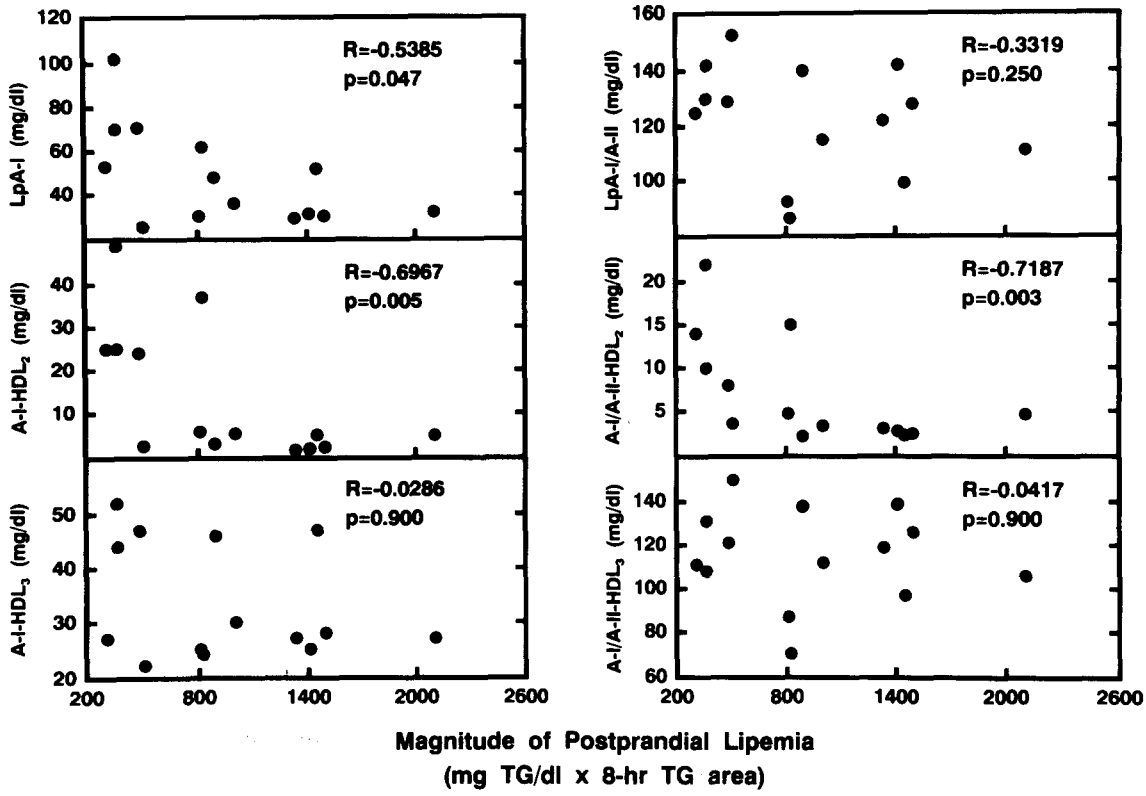

Fig. 2. Relationship between magnitude of postprandial lipemia and plasma levels of LpA-I, A-I-HDL_2, A-I-HDL_3 (left) and LpA-I/A-II, A-I/A-II-HDL_2, and A-I/A-II-HDL_3 (right). R refers to Spearman correlation coefficient.

quantity of this fraction may have been underestimated in our study.

Effective clearance capacity of TGRL after the oral fat load was associated with increased plasma levels of A-I- and A-I/A-II-HDL_2 (Fig. 2). The strong correlation of A-I/A-II-HDL_2 plasma levels with LPL activity as well as the inverse association with HL activity (Table 6) are consistent with previous in vitro experiments showing interconversion of LpA-I/A-II between HDL_2 and HDL_3. Thus, LPL generates redundant surface material from TGRL effecting the anabolic formation of A-I/A-II-HDL_2 (13), while HL appears to convert HDL_2 into HDL_3 by hydrolyzing the core triglycerides acquired from TGRL via CETP (16). Our findings that apoA-I/apoA-II ratios were higher in A-I/A-II-HDL_2 than in A-I/A-II-HDL_3 are at variance with a previous study (40) and suggest that in

TABLE 6. Spearman correlation coefficients among HDL subfraction protein and lipoprotein-modifying enzymes

	Lipoprotein Lipase Activity	Hepatic Lipase Activity	LCAT Activity	CETP Mass
LpA-I	0.504	−0.566	−0.005	−0.688[a]
LpA-I/A-II	0.028	0.322	0.709[b]	−0.665[a]
HDL_2	0.601[a]	−0.832[c]	−0.324	−0.173
A-I-HDL_2	0.559	−0.769[b]	−0.198	−0.232
A-I/A-II-HDL_2	0.692[a]	−0.888[c]	−0.335	0.032
HDL_3	0.115	0.594[a]	0.747[b]	−0.720[b]
A-I-HDL_3	0.091	0.448	0.286	−0.797[b]
A-I/A-II-HDL_3	−0.217	0.580[a]	0.753[b]	−0.433
A-I-HDL_2/A-I-HDL_3	0.496	−0.881[b]	−0.231	−0.141
A-I/A-II-HDL_2/A-I/A-II-HDL_3	0.720[b]	−0.916[c]	−0.511	0.547

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

vivo transformations of HDL_3 into HDL_2 or vice versa are associated with gain or loss of apoA-I. Indeed, recent in vitro studies showed that addition of phosphatidylcholine-apoA-I discs to a lipolytic system containing A-I/A-II-HDL_3 resulted in HDL_2 particles with physicochemical properties closely resembling A-I/A-II-HDL_2 isolated from plasma (G. Miesenboeck and J. R. Patsch, unpublished observation). Furthermore, Clay, Rye, and Barter (42) have shown in in vitro experiments that reduction of HDL size induced by lipolysis of core triglyceride with hepatic lipase resulted in a reduced apoA-I concentration of HDL.

Even though LpA-I and, in particular, A-I-HDL_2 showed inverse associations with the magnitude of postprandial lipemia, the partition of LpA-I between HDL_2 and HDL_3 tended to exhibit weaker associations with lipase activities when compared with the partition of LpA-I/A-II (Table 6). HL appears to prefer A-I/A-II-HDL_2 over A-I-HDL_2 as a substrate as shown by in vitro studies (9) thereby driving the formation of A-I/A-II-HDL_3. However, the differences of A-I-HDL_2 and A-I/A-II-HDL_2 in substrate properties for this enzyme alone are unlikely to explain the differences in the partition of LpA-I and LpA-I/A-II between density fractions. While protein concentrations of LpA-I and LpA-I/A-II were strongly correlated within the HDL_2 density spectrum, their plasma concentration within the HDL_3 density spectrum was not correlated, suggesting selectivity of lipoprotein-modifying enzymes with respect to apolipoprotein content within the HDL_3 range. Indeed, a strong inverse association between A-I-HDL_3 and CETP plasma concentrations (Table 6) was found that suggests distinct influences of CETP on LpA-I and LpA-I/A-II concentrations within the HDL_3 density region. Such a conclusion is consistent with the plasma distribution of CETP, which is primarily associated with LpA-I of small particle size, i.e., HDL_3 and very high density lipoproteins (43). However, the absence of associations between CETP and plasma levels of A-I-HDL_2, A-I/A-II-HDL_2, and A-I/A-II-HDL_3 does not imply a lack of interaction between CETP and these HDL subspecies; rather, other enzymes may be more important for their interconversion and, hence, their plasma levels. Furthermore, we have obtained in vitro evidence that conversion of A-I-HDL_3, but not of A-I/A-II-HDL_3 into particles of HDL_2 size can occur in the absence of lipolytic activity, provided that LCAT, CETP, and triglyceride-rich lipoproteins are present in the incubation mixture (H-O. Mowri, A. Ritsch, J. R. Patsch, H. J. Pownall, and W. Patsch, unpublished observation).

Unlike CETP mass, LCAT activity displayed a strong association with A-I/A-II-HDL_3. Since A-I-HDL_3 is thought to be the preferred substrate for LCAT (44), the positive association of LCAT activity with A-I/A-II-HDL_3 plasma concentrations would be consistent with interconversion of A-I-HDL_3 into A-I/A-II-HDL_3 as a result of LCAT action. Alternatively, cholesteryl esters, generated by LCAT, may transfer from A-I-HDL_3 to A-I/A-II-HDL_3 as suggested by Ohta et al. (44), which may effect the plasma concentration of this lipoprotein subspecies. The lack of associations between LCAT activity and HDL_2 subfractions is consistent with low reactivity of LCAT with HDL_2 as compared with HDL_3 (45, 46).

Turnover studies in humans on high fat diets showed an excellent inverse correlation of apoA-I plasma levels with the fractional catabolic rate of apoA-I, suggesting that the differences in levels of apoA-I result from differences in HDL catabolism (47). Since our study was conducted under strict dietary control, differences in LpA-I levels were perhaps due to different rates of apoA-I catabolism (47). As plasma levels of LpA-I in HDL_2 showed the largest variation between individuals, the ability to form this HDL subpopulation and/or to reduce its catabolism is probably of key importance in maintaining high levels of LpA-I.

The differences in the associations of LpA-I and LpA-I/A-II with CAD are poorly understood, although a number of concepts have been presented. A recent study in Japanese subjects (48) showed that apoA-I binds and stabilizes prostacyclin, which would be expected to reduce thrombus formation and vasoconstriction. However, Tsai et al. (49) were unable to confirm these results. Particles containing only apoA-I may be the physiologic acceptor of cellular cholesterol (8), but this small-sized LpA-I represents only a minor fraction of plasma LpA-I and can thus not directly account for the inverse association between plasma levels of LpA-I and CAD. In vitro studies showed that LpA-I, but not LpA-I/A-II enhances cholesterol efflux from mouse adipocytes. In fact, LpA-I/A-II may inhibit the LpA-I-stimulated efflux from cholesterol (50). However, in a number of other mammalian cells including rabbit aortic smooth muscle cells (51) and bovine aortic endothelial cells (52), LpA-I and LpA-I/A-II appear to function equally well in removing cholesterol. Furthermore, we did not observe a difference between A-I- and A-I/A-II HDL_2 or between A-I- and A-I/A-II-HDL_3 to promote cholesterol efflux from cholesterol-loaded $P388D_1$ macrophages (J. R. Patsch and W. Patsch, unpublished observation). The cholesterol-mobilizing ability unique to LpA-I in adipocytes may thus relate to a specific pathway in adipocytes. However, the possibility cannot be excluded that the preferential association of LCAT and CETP with LpA-I enhances its ability to promote cholesterol efflux in vivo (44).

Alternatively, the inverse association of LpA-I with CHD may in part reflect its inverse association with the magnitude of postprandial lipemia described in this study. Accordingly, high levels of LpA-I may be indicative of a metabolic state, i.e., effective removal of triglyceride-rich lipoproteins which confers protection from CAD. Two recent studies in patients with angiographically verified

CAD and controls showed that markers of the postprandial metabolism of TGRL are useful in discriminating cases from controls (53, 54). In one of these studies, postprandial plasma triglyceride values were at least as accurate as HDL cholesterol levels in identifying cases and controls (54). While these studies argue for a direct relationship between TGRL or their remnants with CHD, they do not exclude the possibility that certain HDL particles directly interfere with the atherogenic process. It is thus conceivable that triglyceride metabolism plays a role in establishing type and quantity of HDL particles with specific ability to interact and/or return cholesterol from cells of the arterial wall.

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