Altered particle size distribution of apolipoprotein A-I-containing lipoproteins in subjects with coronary artery disease

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Abstract Plasma high density lipoproteins (HDL) can be separated into two subpopulations of apolipoprotein A-I-containing particles: those that also contain apoA-II [Lp(AI w AII)] and those that do not [Lp(AI w/o AII)]. These particles were isolated by immunoaffinity chromatography from 17 men (9 normolipidemic (NL), 8 hyperlipidemic (HL)) with symptomatic coronary artery disease (CAD), from 17 NL men without any symptoms of CAD (healthy controls), and from 10 NL men with entirely normal coronary arteriograms (CAD-free controls). The distributions of particle size in these two subpopulations were determined by gradient gel electrophoresis and densitometric scanning. Approximately half of the Lp(AI w AII) particles in all subjects were distributed in the 8.2–9.2 nm interval. For patients with CAD, a greater fraction of the particles were small, in the 7.0–8.2 nm interval [33% in CAD vs. 26% in CAD-free controls ($P<0.01$) and 19% in healthy controls ($P<0.0001$)], and a smaller fraction of the particles were in the 9.2–11.2 nm interval (14% in CAD vs. 24% in CAD-free control ($P<0.002$) and healthy control groups ($P<0.0001$)). The Lp(AI w/o AII) of both control groups were primarily composed of two discrete subpopulations in the 8.2–9.2 nm and the 9.2–11.2 nm intervals. In CAD patients there were fewer particles in the 9.2–11.2 nm size interval (23% in CAD vs. 33% in CAD-free controls ($P<0.005$) and 36% in healthy controls ($P<0.0001$)), and more particles in the smallest 7.0–8.2 nm size interval (32% in CAD vs. 23% in CAD-free controls ($P<0.01$) and 18% in healthy controls ($P<0.0001$)). Thus, the spectrum of HDL particle sizes in patients with CAD tends to be shifted toward the smaller particle when compared with the two control groups. This was observed in both NL and HL patients with HDL cholesterol (CH) values in the normal range. As a group, CAD patients had lower HDL (42 ± 7 mg/dl) and HDL_2 (6 ± 4 mg/dl) CH than healthy (HDL: 49 ± 7, HDL_2: 12 ± 6 mg/dl) and CAD-free (HDL: 51 ± 9, HDL_2: 12 ± 6 mg/dl) controls. When controls and patients were compared for their frequencies of abnormal HDL CH levels and particle sizes, abnormalities in HDL and HDL_2 CH levels were not significantly more frequent (twofold) among CAD patients than among controls. However, an abnormally increased fraction of 7.0–8.2 nm particles and a reduced fraction of 9.2–11.2 nm particles were 5- to 11-times more frequent among patients ($P<0.05$ to 0.0001). Thus, using this unique method for segregating HDL particles, the presence of CAD is found to be more strongly associated with abnormalities in HDL particle size than with low HDL CH levels. These observations, although preliminary, suggest a more effective means of detecting subjects at risk for CAD. —**Cheung, M. C., B. G. Brown, A. C. Wolf, and J. J. Albers.** Altered particle size distribution of apolipoprotein A-I-containing lipoproteins in subjects with coronary artery disease. *J. Lipid Res.* 1991. **32:** 383–394.

Supplementary key words HDL subpopulations • HDL particle size • coronary artery disease • immunoaffinity chromatography • gradient polyacrylamide gel electrophoresis

Early studies in the 1950s using ultracentrifugal methods demonstrated the association of a decreased level of high density lipoproteins (HDL) with coronary artery disease (CAD) (1, 2). This observation has since been extended for both the apolipoprotein (apo) A (A-I and/or A-II) and cholesterol components of HDL (3, 4). However, the metabolic basis for this association is still unknown. Human HDL is a heterogeneous population of particles that differ in their physical (density, size, and charge) and chemical (protein and lipid composition) properties. Conventionally, they are divided into two subfractions: HDL_2 ($F^o_{1.20}$ 3.5–9.0, d 1.063–1.125 g/ml) and HDL_3 ($F^o_{1.20}$ 0–3.5, d 1.125–1.21 g/ml). Based on this classification, it has been reported that the relative proportions of these two HDL subfractions and, in some cases, their chemical compositions differ among NL and HL individuals and individuals with diabetes mellitus, thyroid, liver, and kidney disorders, and CAD (5–12).

Affinity columns containing antibodies specific for apoA-I and A-II provide an alternative method for studying HDL (13). With this technique, we have isolated and characterized two populations of HDL particles from healthy NL subjects: particles containing both A-I and A-

Abbreviations: CAD, coronary artery disease; HDL, high density lipoprotein; NL, normolipidemic; HL, hyperlipidemic; Lp(AI w AII), HDL particles containing apoA-I and apoA-II; Lp(AI w/o AII), HDL particles containing apoA-I without apoA-II; CH, cholesterol; TG, triglyceride; gPAGE, gradient polyacrylamide gel electrophoresis.

II [Lp(AI w AII)] and particles containing A-I but no A-II [Lp(AI w/o AII)]. Together, they represent nearly all HDL particles. In normolipidemic (NL) subjects 25–50% of plasma apoA-I is found in Lp(AI w/o AII). Each of these two subpopulations is heterogeneous in size and is found in both HDL_2 and HDL_3 (13, 14). The particle size distributions of these two subpopulations in various disease states have yet to be examined. The present study focuses on the characteristics of these particles in patients with CAD. We find that the relative proportion of Lp(AI w AII) to Lp(AI w/o AII) in CAD patients is comparable to that in healthy NL subjects and subjects with normal coronary arteriograms. However, the particle size distributions of these two HDL subpopulations in CAD patients differ significantly from those in the control subjects.

METHODS

Subjects

Seventeen male patients (age 26–60) with symptomatic CAD who demonstrated at least 50% occlusion in one or more vessel(s) by coronary angiography at the University of Washington Medical Center were recruited for this study. Eight hypercholesterolemic (>90th percentile) patients were consecutive entrants in a lipid-lowering trial; the other 9 were selected from a database of catheterized patients as an NL CAD population that matched the hyperlipidemic (HL) group in age (<60), sex, history of hypertension, and CAD severity (NV50). Only 4 of the 17 patients were smokers. HL patients were not on hypolipidemic therapy at the time their blood was drawn. Two groups of control subjects were recruited for this study. The first group consisted of 10 men (age 38–63) with atypical chest pain, eventually diagnosed as noncardiac after catheterization. They had entirely normal coronary arteriograms (CAD-free control). One of them was a smoker. The second control group consisted of 17 apparently healthy NL men (age 26–69), nonsmokers without any symptoms of CAD or history of alcoholism, diabetes mellitus, kidney, liver, or thyroid disorders (healthy controls). Venous blood was drawn into EDTA-containing Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) after a 12–14 h overnight fast, at least 2 months after any clinical event or cardiac catheterization. Plasma was promptly separated by low-speed centrifugation at 4°C and immediately used for isolation of apoA-I-containing lipoproteins.

Isolation of A-I-containing lipoproteins

ApoA-I-containing lipoproteins with or without A-II were isolated according to a previously established two-step immunoaffinity chromatography method (13, 15). Specifically, to isolate Lp(AI w AII), 4 ml of plasma from each subject was incubated with 30 ml of anti-A-II immunosorbent for 1 h at 4°C. Nonbinding plasma proteins free of A-II eluted from the anti-A-II immunosorbent were incubated with 15 ml of anti-A-I immunosorbent for 1 h at 4°C to isolate Lp(AI w/o AII). In each case, the lipoproteins bound to the immunosorbents were rapidly dissociated from the antibodies with 3 M NaSCN in 0.02 M sodium phosphate buffer, pH 7.0, and immediately filtered through a Sephadex G-25 (Pharmacia LKB Biotechnology, Piscataway, NJ) column to remove the thiocyanate. This isolation procedure has been documented to have no detectable quantitative or qualitative effect on HDL subpopulations of NL and HL subjects studied by gradient polyacrylamide gel electrophoresis (gPAGE) (16). Recoveries of A-I and A-II from the antibody columns were comparable between NL and HL subjects, 95% for A-I and 90% for A-II (16).

Gradient polyacrylamide gel electrophoresis

The size subpopulations of Lp(AI w AII) and Lp(AI w/o AII) particles were separated by nondenaturing gradient polyacrylamide gel electrophoresis (gPAGE) using precast 4–30% gradient gels (Pharmacia LKB). Routinely, lipoprotein particles containing approximately 10–20 μg of A-I were applied to each sample well. High molecular weight calibration protein mixture (HMW Calibration Kit, Pharmacia LKB) was included in each gel run. Electrophoresis was carried out in 0.09 M Tris, 0.08 M borate, 0.003 M EDTA, pH 8.35, at 10°C at 125 V for 24 h. Gels were stained for protein overnight with 0.04% Coomassie Brilliant Blue G-250 dissolved in 3.5% perchloric acid, destained in 5% acetic acid until background was clear, and scanned by a laser densitometer (Pharmacia LKB). Repeat isolations and analysis of plasma from several different subjects were performed to determine the reproducibility of the isolation and gPAGE analysis methods and physiological variation of HDL subpopulations.

Calculation of particle size and distribution

Particle sizes of the various subpopulations of Lp(AI w AII) and Lp(AI w/o AII) were determined from their migration distance in reference to the calibration proteins. A standard curve of relative migration distance versus Stokes diameters was generated for each electrophoresis run. The hydrated Stokes diameters of the calibration proteins were: thyroglobulin (17 nm), apoferritin (12.2 nm), lactate dehydrogenase (8.2 nm), and bovine albumin (7.1 nm). Based on the clustering of particle sizes of healthy, NL subjects, four size intervals (7.0–8.2 nm, 8.2–9.2 nm, 9.2–11.2 nm, and 11.2–17.0 nm) were adopted to describe the size subpopulation profiles of Lp(AI w AII) and Lp(AI w/o AII), and to facilitate comparison among individuals. To calculate the percentage of particles within each size interval, the total integrated area of

each densitometric scan between 7.0 and 17.0 nm (the largest and smallest reference proteins) was considered to be 100%. Perpendicular lines were dropped from positions corresponding to the Stokes diameters 7.0, 8.2, 9.2, 11.2, and 17.0 nm. The ratio of the area between two perpendicular lines to the total area of the scan represents the relative proportion of each size subpopulation in Lp(AI w AII) or Lp(AI w/o AII). All calculations were performed with the LKB 2400 Gelscan XL® software.

Lipid and apolipoprotein analysis

Cholesterol (CH) was analyzed by the enzymic methods of Cooper et al. (17). Triglyceride (TG) was analyzed by Agent® enzymic kit (Abbott), which includes reagents for quantitating free glycerol. Determination of both CH and TG were performed on an ABA-200 analyzer (Abbott, Dallas, TX). Cholesterol in HDL, HDL_2, and HDL_3 was determined by a two-step dextran sulfate-magnesium precipitation method (18). ApoA-I, A-II, and B were analyzed by previously established specific immunoassays (19–21). Distribution of plasma apoA-I between Lp(AI w AII) and Lp(AI w/o AII) was calculated as described (13).

Statistical analyses

In view of the small sample size, the Mann-Whitney U test for nonparametric analyses was used throughout the study (22). Computation was performed with the SPSS/PC™ software. All significance levels given are those for a two-tailed test.

RESULTS

Lipoprotein lipids and apoproteins

The lipid, apoA-I, A-II, and B concentrations of the three groups of subjects are shown in **Table 1** and **Table 2**. Three CAD patients (nos. 5, 7, and 8, Table 2) had elevated plasma CH and TG (>90th percentile), five (nos. 1–4 and 6) had elevated plasma CH, and the remaining nine (nos. 9–17) were NL (<90th percentile) according to the Lipid Research Clinic Prevalence Study data (23). Despite the significant differences in their plasma CH and TG, and LDL CH, the apoA-I, and HDL, HDL_2, and HDL_3 CH concentrations of NL and HL CAD patients were comparable (**Table 3**). However,

TABLE 1. Lipid and apolipoprotein profile of healthy normolipidemic subjects and CAD-free subjects

		Plasma Lipids		Apolipoproteins			Lipoprotein Cholesterol				A-I in	
Subject	Age	CH	TG	A-I	A-II	B	LDL	HDL	HDL_2	HDL_3	Lp (AI w AII)	Lp (AI w/o AII)
	yr							*mg/dl*				
Healthy												
1	44	212	166	110	30	107	144	34	4	30	74	36
2	60	212	85	126	32	143	154	49	11	38	82	44
3	69	211	59	166	30	110	144	55	19	36	115	51
4	39	210	109	151	26	144	141	47	8	39	103	48
5	43	188	72	142	24	93	119	55	12	43	92	50
6	57	187	42	168	28	98	124	55	17	38	108	60
7	60	185	94	126	32	94	119	47	11	36	81	45
8	42	185	75	123	24	107	121	49	14	35	69	54
9	46	183	79	129	21	76	124	43	7	36	93	36
10	49	178	58	111	32	74	99	60	25	35	74	37
11	36	177	55	122	26	104	114	52	13	39	77	45
12	60	176	79	126	22	113	117	43	10	33	79	47
13	31	176	73	137	25	98	110	51	12	39	92	45
14	27	170	114	115	29	92	111	36	3	33	70	45
15	40	155	38	126	44	74	89	59	18	41	78	48
16	33	146	106	149	28	68	73	52	9	43	88	61
17	26	142	88	142	25	76	82	42	7	35	71	71
CAD-free												
1	53	230	205	125	29	126	159	39	3	36	85	40
2	63	225	72	162	42	118	143	62	16	46	83	79
3	57	225	78	158	40	120	153	63	21	42	111	47
4	50	220	36	134	24	106	156	57	14	43	86	48
5	43	213	112	130	30	123	149	50	11	39	82	48
6	42	205	62	124	25	105	144	52	11	41	86	38
7	38	194	130	109	26	83	119	39	3	36	86	23
8	37	173	85	125	30	95	115	43	6	37	95	30
9	48	171	48	133	31	80	104	55	18	37	77	56
10	46	169	72	124	26	92	112	46	12	34	78	46

CH, cholesterol; TG, triglycerides; LDL, low density lipoproteins; HDL, high density lipoproteins.

TABLE 2. Lipid and apolipoprotein profile of subjects with CAD

Subject[a]	Age	NV-50[b]	Plasma Lipids		Apolipoproteins			Lipoprotein Cholesterol				A-I in	
			CH	TG	A-I	A-II	B	LDL	HDL	HDL_2	HDL_3	Lp (AI w AII)	Lp (AI w/o AII)
	yr							*mg/dl*					
Hyperlipidemic													
$1^{T,F}$	45	2	392	204	118	33	286	317	40	5	35	80	38
2	38	2	344	95	124	25	191	282	42	4	38	68	56
3^{B}	43	1	313	160	130	28	187	234	50	11	39	87	43
4	26	1	295	73	104	28	166	232	44	5	39	60	44
5^{B}	58	3	285	256	146	35	168	189	44	4	40	93	53
6	42	2	266	170	124	29	138	180	38	2	36	77	47
7^{B}	47	1	264	323	123	33	152	152	38	5	33	71	52
8	37	2	255	509	129	25	145	153	31	2	29	83	46
Normolipidemic													
9^{B}	55	1	249	174	117	25	144	176	34	4	30	77	50
10	60	1	223	137	121	24	131	161	38	3	35	92	47
11^{B}	48	2	209	127	127	24	110	135	48	9	39	76	43
12	49	3	203	102	139	25	104	139	44	5	39	79	37
13	43	3	181	68	119	28	99	129	54	9	45	85	66
$14^{B,T}$	59	2	164	96	116	23	85	102	33	5	28	86	52
15	55	3	160	39	149	25	83	107	57	17	40	85	27
$16^{B,F}$	54	3	142	94	138	32	76	83	40	9	31	63	54
17	54	3	135	63	112	26	67	77	40	9	31	90	31

[a]Subjects on medication are identified by the superscripts B, F, and T for beta adrenergic blocker, furosemide, and thiazide, respectively.
[b]NV-50 = number of vessels with at least 50% occlusion.

HL CAD patients had higher A-II levels than the NL CAD patients ($P<0.05$). As a group, CAD patients had significantly higher plasma CH ($P<0.02$), TG ($P<0.01$), apoB ($P<0.02$), and LDL CH ($P<0.01$), but significantly lower HDL and HDL_2 CH ($P<0.01$ and 0.002, respectively) than the healthy controls. Similar differences were observed between CAD patients and CAD-free controls, but reached statistical significance only in HDL and HDL_2 CH ($P<0.03$). In all groups of subjects, HDL_3 CH levels were similar.

TABLE 3. Mean and standard deviation of lipid and apolipoprotein profile of subjects

Subject	Age	Plasma Lipids		Apolipoproteins			Lipoprotein Cholesterol				A-I in	
		CH	TG	A-I	A-II	B	LDL	HDL	HDL_2	HDL_3	Lp (AI w AII)	Lp (AI w/o AII)
	yr						*mg/dl*					
Healthy												
Mean	45	182	82	133	28	98	117	49	12	37	85	48
± SD	13	21	31	17	5	22	22	7	6	4	14	9
CAD-free												
Mean	48	202	90	132	30	105	135	51	12	39	87	45
± SD	8	24	49	16	6	17	21	9	6	4	10	15
Hyperlipidemic												
Mean	42	302	224	125	30	179	217	41	5	36	78	47
± SD	9	47	141	12	4	47	60	6	3	4	11	6
Normolipidemic												
Mean	53	185	100	126	26	100	123	43	8	35	81	45
± SD	5	38	41	13	3	25	34	8	4	6	9	12
All CAD												
Mean[a]	48	240^{1}	158^{2}	126	27	137^{1}	168^{2}	$42^{1,y}$	$6^{3,y}$	36	80	46
± SD	9	73	116	12	4	54	67	7	4	5	10	9

[a]Significantly different from healthy control at $^{1}P < 0.02$, $^{2}P < 0.01$, $^{3}P < 0.002$; by Mann-Whitney U test. Significantly different from CAD-free control at $^{y}P < 0.03$; by Mann-Whitney U test.

In plasma, apoA-I is distributed between Lp(AI w AII) and Lp(AI w/o AII). In this study, 64 ± 5% (range 50–72%), 66 ± 8% (range 51–79%), and 63 ± 6% (range 54–76%) of plasma A-I was associated with Lp(AI w AII) in healthy controls, CAD-free controls, and CAD patients, respectively. The remaining plasma A-I was associated with Lp(AI w/o AII). Hence the distribution of plasma A-I between these two populations of particles was comparable for all three groups. Likewise, their concentrations were similar (Table 3).

Lp(AI w AII) particle sizes

When Lp(AI w AII) and Lp(AI w/o AII) particles were separated by gPAGE and stained for protein, numerous size subpopulations could be seen. Representative scans depicting the various size subpopulations of Lp(AI w AII) and Lp(AI w/o AII) of controls and CAD patients are shown in **Fig. 1**. In all subjects, approximately half of the Lp(AI w AII) particles were found in the 8.2–9.2 nm size interval. However, the Stokes diameter corresponding to the peak position within that region was smaller in CAD patients (8.64 ± 0.17 nm) than in healthy (8.85 ± 0.17 nm, $P<0.013$) and CAD-free controls (8.86 ± 0.15 nm, $P<0.004$) (Fig. 1, left panel). The factions of particles in the 7.0–8.2 nm and 9.2–11.2 nm regions were different between CAD patients and the two control groups. Specifically, CAD patients had more particles in the 7.0–8.2 nm region and fewer particles in the 9.2–11.2 nm region. **Fig. 2** suggests that CAD patients can be distinguished from normals by a greater fraction of particles in the 7.0–8.2 nm and a lower fraction in the 9.2–11.2 nm range. We compared these two study populations (control and CAD) for their frequencies of abnormal HDL CH and abnormal particle sizes. Here abnormal was defined as roughly 10th or 90th percentile cutoff values for the control population (<37 mg/dl HDL CH, <4 mg/dl HDL_2 CH (Table 1); >28% of Lp(AI w AII) or 30% of Lp(AI w/o AII) in the 7.0–8.2 nm range; and <14% of Lp(AI w AII) or <21% of Lp(AI w/o AII) in the 9.2–11.2 nm range) (Fig. 2). HDL CH was abnormal in 2 of 27 (7.4%) of the controls and 3 of 17 (17.6%) CAD patients (Table 2). The relative frequencey of abnormally low HDL CH between CAD patients and controls (17.6%/7.4%) was 2.4. The odds ratio relating CAD patients to controls was 2.7, with $P>0.5$ by chi square analysis (24). Likewise, increased amounts of small (7.0–8.2 nm) Lp(AI w AII) occurred in 2 of 27 (7.4%) of the controls and 14 of 17 (82%) with CAD (relative frequency 11.1; odds ratio 58.3; $P<0.0001$). Reduced amounts of the larger (9.2–11.2 nm) Lp(AI w AII) particles were present in 2 of 27 (7.4%) of controls and 12 of 17 (70.6%) with CAD (relative frequency 9.5; odds ratio 30.0; $P<0.0001$) (Fig. 2). Thus in comparison of sex- and age-matched control and CAD groups, particle size abnormalities in CAD subjects are 9- to 11-fold more frequent than in controls. In all three groups of subjects, only 5–6% of all Lp(AI w AII) particles had Stokes diameters greater than 11.2 nm.

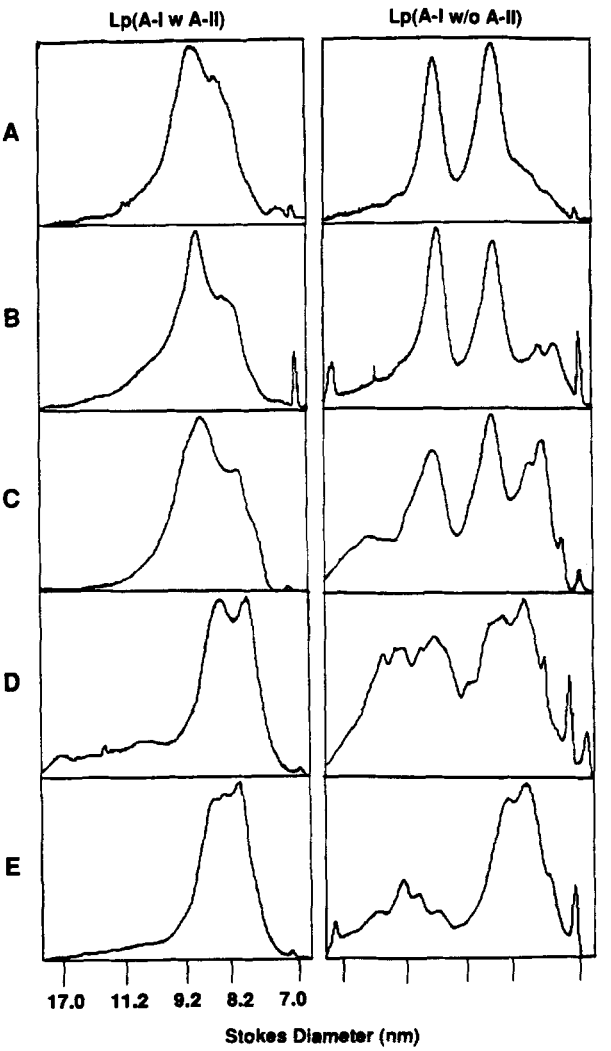

Fig. 1. Representative densitometric scans of 4–30% gradient gel electrophoresis of Lp(AI w AII) (left panel) and Lp(AI w/o AII) (right panel) of A) normolipidemic healthy controls (No. 2, Table 1), and B) CAD-free controls (No. 10, Table 1). Scans C–E are those of CAD patients with normal lipid levels, elevated plasma cholesterol, and elevated plasma cholesterol and triglyceride (Nos. 13, 4, and 5, Table 2), respectively.

Lp(AI w/o AII) particle sizes

In the two control groups, Lp(AI w/o AII) contained mostly particles of two discrete sizes in the 8.2–9.2 nm, and 9.2–11.2 nm intervals (Fig. 1A, B, right panel). The hydrated Stokes diameters corresponding to the peak position in these two size regions were comparable in the two control groups, being 8.56 ± 0.12 nm and 10.22 ± 0.15 nm, respectively. In CAD patients, the peak scan position in the 8.2–9.2 nm interval was slightly but significantly smaller: 8.35 ± 0.08 nm ($P<0.001$). This was not the case with particles in the 9.2–11.2 nm range.

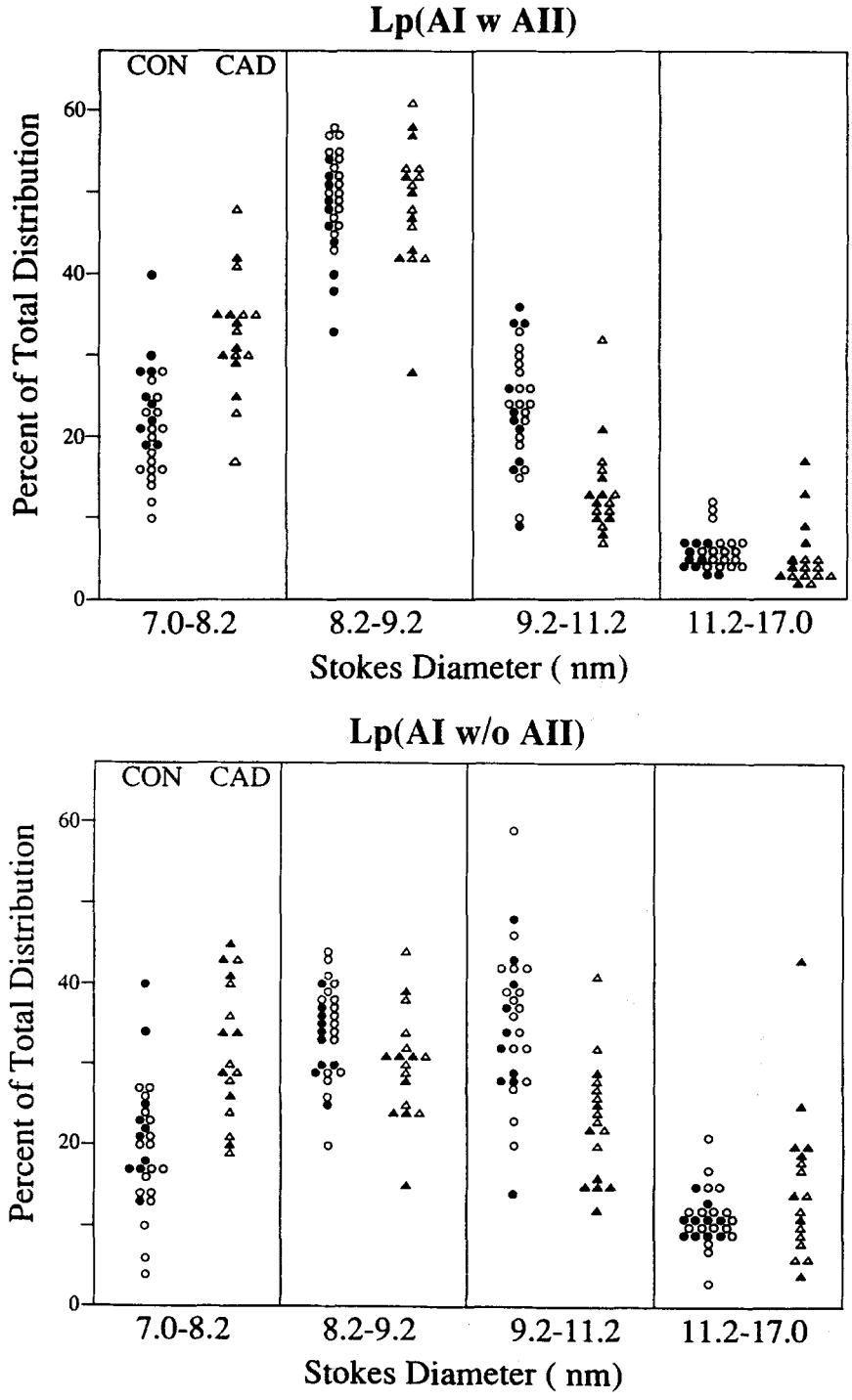

Fig. 2. Abnormalities of HDL particle size distribution in CAD patients. Percent of total particle distribution in four Stokes diameter (nm) intervals. Top: Lp(AI w AII); bottom: Lp(AI w/o AII). (○) Healthy, normolipidemic controls; (●) CAD-free controls; (△) normolipidemic CAD patients; and (▲) hyperlipidemic CAD patients.

The particle size distribution of this HDL subpopulation differed between patients and controls. While the proportion of particles in the 8.2–9.2 nm size interval in CAD patients (30.0 ± 6.7%) was only slightly lower than CAD-free controls (32.9 ± 4.4%) and healthy controls (34.7 ± 6.4%), particles in the 9.2–11.2 nm interval were substantially reduced in the patients. This was more prominent in HL subjects (**Table 4** and Fig. 2). These reductions were accompanied by reciprocal increases in particles smaller than 8.2 nm in both NL and HL CAD

TABLE 4. Size subpopulation distribution (%) of Lp (AI w AII) and Lp (AI w/o AII)

Subjects	Size Interval (nm) 7.0–8.2	8.2–9.2	9.2–11.2	11.2–17.0
	%			
Lp (AI w AII)				
Healthy controls (17)	18.9 ± 5.3	51.2 ± 4.5	23.5 ± 6.1	6.4 ± 2.4
CAD-free controls (10)	25.6 ± 6.4	45.5 ± 6.7	23.8 ± 8.8	5.1 ± 1.6
CAD patients (17)	32.5 ± 7.3[b,1]	48.5 ± 7.8	13.5 ± 5.9[b,3]	5.4 ± 4.1[a]
Normolipidemic (9)	32.4 ± 9.1	49.8 ± 6.0	14.2 ± 7.4	3.6 ± 1.0
Hyperlipidemic (8)	32.6 ± 5.1	47.1 ± 9.7	12.8 ± 4.0	7.5 ± 5.2
No Rx (9)	33.0 ± 7.9	47.0 ± 9.6	13.9 ± 7.1	6.1 ± 5.2
On Rx (8)	32.0 ± 7.1	50.2 ± 5.4	13.1 ± 4.5	4.6 ± 2.3
Lp (AI w/o AII)				
Healthy controls (17)	17.6 ± 6.9	34.7 ± 6.5	36.2 ± 9.2	11.4 ± 4.1
CAD-free controls (10)	23.0 ± 8.3	32.9 ± 4.4	33.3 ± 9.5	10.8 ± 2.0
CAD patients (17)	31.9 ± 8.5[b,1]	30.0 ± 6.7[a]	23.1 ± 7.4[b,2]	15.1 ± 9.3
Normolipidemic (9)	30.0 ± 8.3	31.9 ± 6.2	27.0 ± 6.3	11.1 ± 4.4
Hyperlipidemic (8)	34.0 ± 8.8	27.9 ± 7.1	18.6 ± 6.0*	19.5 ± 11.5*
No Rx (9)	33.7 ± 7.4	30.8 ± 5.7	21.4 ± 6.3	14.1 ± 6.8
On Rx (8)	29.9 ± 9.7	29.1 ± 8.1	24.9 ± 8.5	16.1 ± 11.9

Numbers in parentheses indicate sample size; all other values represent mean ± SD. Rx, ongoing therapy with thiazide diuretics or furosemide and/or with beta adrenergic blocking drugs.

Significant differences (by Mann-Whitney U test) between CAD patients and healthy controls are indicated with the letters *a* ($P < 0.05$) and *b* ($P < 0.0001$).

Significant differences between CAD patients and CAD-free controls are indicated with the numbers *1* ($P < 0.01$), *2* ($P < 0.005$), and *3* ($P < 0.002$).

Significant differences between normolipidemic and hyperlipidemic CAD patients are indicated by *.

patients. As above, increased amounts of small Lp(AI w/o AII) occurred in 2 of 27 (7.4%) controls and 8 of 17 (47.0%) with CAD (relative frequency 6.4; odds ratio 11.1; $P = 0.007$), and reduced amounts of the larger particles in 2 of 27 (7.4%) of controls, and 6 of 17 (35.3%) with CAD (Fig. 2) (relative frequency 4.8; odds ratio 6.8; $P = 0.053$). In HL but not NL CAD patients, relatively more particles larger than 11.2 nm were also detected.

Normolipidemic versus hyperlipidemic CAD

Statistical analysis showed that NL CAD patients and HL CAD patients did not differ significantly in the subpopulation distribution of Lp(AI w AII) (Table 4). Hence, the shift of Lp(AI w AII) distribution observed in CAD patients was independent of their plasma lipid profile. When the subpopulation distribution of Lp(AI w/o AII) of NL CAD patients was compared with HL CAD patients, HL CAD patients had a significantly lower percentage of particles in the 9.2–11.2 nm interval ($P<0.02$) and significantly higher percentage of particles larger than 11.2 nm ($P<0.05$). No significant differences were found with the other subpopulations (Table 4 and Fig. 2). Thus, in Lp(AI w/o AII), the decrease of particles in the 9.2–11.2 nm interval and the increased presence of particles larger than 11.2 nm may be related to hyperlipidemia.

Medications

In order to explore the possible contribution of medications known to alter serum lipid levels (25–27) we determined all ongoing medications from patient interview and chart review. Three patients with CAD were taking diuretics (thiazide or furosemide); seven were taking beta adrenergic blocking agents (Table 2). Serum lipid values were not significantly different in the eight CAD patients taking these medications from the nine patients not taking them (CH 252 ± 81 mg/dl vs. 229 ± 67 mg/dl; TG: 179 ± 80 mg/dl vs. 140 ± 144 mg/dl). Table 4 indicates that the particle size distributions of Lp(AI w AII) and Lp(AI w/o AII) were also not affected by these medications.

Variation in HDL particle size distribution between analyses and within individuals

To determine the extent technical and physiological variation may contribute to the observed particle size differences between CAD patients and controls, we examined the reproducibility of the immunoaffinity isolation and gPAGE analytical procedures used in this study. Lp(AI w AII) and Lp(AI w/o AII) were isolated twice or thrice from eight plasma samples with CH levels between 159 and 581 mg/dl, and TG levels between 39 and 4104 mg/dl. **Table 5** shows that the particle size profiles of HDL subpopulations of repeat isolations from the same plasmas were quite comparable, with a mean difference of 1–3% distribution in all size intervals.

Physiological variation was studied by comparing the HDL particle size profiles of blood samples obtained from eight free-living individuals at two different times. The mean differences in plasma CH and TG levels between

TABLE 5. Comparison of particle size profiles (% distribution) of Lp (AI w AII) and Lp (AI w/o AII) obtained by repeat isolations from same plasmas

			Size Interval (nm)							
			Lp (AI w AII)				Lp (AI w/o AII)			
Plasma	CH	TG	7.0-8.2	8.2-9.2	9.2-11.2	11.2-17.0	7.0-8.2	8.2-9.2	9.2-11.2	11.2-17.0
	mg/dl					%				
1	159	49	15[a]	54	24	7	11	37	42	10
			15	54	24	7	10	33	43	14
2	167	39	11	55	25	9	12	39	38	11
			12	56	24	8	13	34	36	17
3	177	55	10	52	32	6	13	35	42	10
			12	49	31	8	12	33	44	11
4	180	74	27	52	18	3	21	30	36	13
			25	51	17	7	19	34	35	12
5	210	133	20	58	20	2	17	40	34	9
			22	54	20	4	22	36	32	10
			25	57	16	2	18	35	36	11
6	229	96	20	45	29	6	15	42	36	7
			17	50	29	4	16	44	34	6
7	249	78	11	55	29	5	10	35	38	17
			9	61	25	5	13	34	37	16
8[b]	581	4104	13	18	59	10	27	9	49	15
			12	18	60	10	35	8	40	17
			11	18	61	10	31	7	41	21
Difference[c] Mean ± SD			2 ± 1	2 ± 2	1 ± 2	1 ± 1	3 ± 2	3 ± 2	3 ± 3	2 ± 2

[a]Numbers in each row represent particle size profile of an isolation.
[b]Plasma of a subject with hepatic triglyceride lipase deficiency.
[c]Observed difference between the second or third isolation and the first isolation.

the two samples of each individual were 19 and 22 mg/dl, respectively (**Table 6**). Differences in percent distribution within a size interval ranged from 0 to 9 with a mean difference of about 2-4%. These differences reflect not only physiological variation but also variation between isolations and gel analysis. These data indicate that the observed particle size differences between CAD patients and controls (Table 4) could not be entirely attributed to methodological and physiological variation.

DISCUSSION

We have isolated two types of apoA-I-containing lipoprotein particles from the plasma of 17 patients with coronary artery stenosis, 10 control subjects with arteriographically normal coronary arteries, and 17 healthy NL subjects without CAD, and have determined their particle size distribution by gPAGE. We found that in both populations of A-I-containing lipoproteins there were significantly fewer particles in the 9.2-11.2 nm region, and significantly more particles in the 7.0-8.2 nm region. These changes occurred in both NL and HL CAD patients, whose HDL CH concentrations were in the normal to low-normal range (Table 4 and Fig. 2). Thus, there was a preponderance of small HDL particles associated with CAD. Although HDL subpopulation particle size was altered in association with CAD, the concentrations of Lp(AI w AII) and Lp(AI w/o AII) in CAD patients were comparable with the controls. This is inconsistent with an earlier report that showed the CAD patients had lower Lp(AI w/o AII) levels than controls (28). This discrepancy is possibly due to population differences and/or differences in the immunochemical methods used to quantitate apoA-I in the two apo-specific HDL subpopulations.

The analysis of HDL polydispersity by gradient polyacrylamide gel electrophoresis was first reported by Blanche et al. (29). These investigators demonstrated that HDL materials in the size intervals of 9.7-12.9 nm, 8.8-9.7 nm, and 7.2-8.8 nm approximate the HDL_{2b}, HDL_{2a}, and HDL_3 components defined by ultracentrifugal methods. Thus, it appears that the Lp(AI w AII) and Lp(AI w/o AII) particles of CAD patients are enriched with dense HDL_3 components such as the HDL_{3D} characterized by zonal ultracentrifugation (30), and are deficient in the

TABLE 6. Comparison of particle size profiles (% distribution) of Lp (AI w AII) and Lp (AI w/o AII) isolated from the plasmas of eight individuals obtained at different times

Subject Plasma	Sampling Intervals	CH	TG	Size Interval (nm) Lp (AI w AII) 7.0–8.2	8.2–9.2	9.2–11.2	11.2–17.0	Lp (AI w/o AII) 7.0–8.2	8.2–9.2	9.2–11.2	11.2–17.0
	months		*mg/dl*					%			
1-1		147	43	15	53	27	5	8	32	53	7
2	3	132	42	13	58	24	5	10	32	51	7
2-1		148	36	10	59	27	4	9	44	43	4
2	21	182	69	13	56	20	11	6	39	45	10
3-1		176	38	13	59	23	5	11	40	40	10
2	18	133	45	11	54	30	5	15	31	44	10
4-1		182	72	18	53	23	6	22	40	27	11
2	2	180	137	23	57	17	3	24	42	25	9
5-1		210	95	22	53	21	4	22	42	28	7
2	4	173	79	24	51	21	4	25	33	32	11
6-1		230	78	17	48	31	4	16	20	48	15
2	48	235	66	17	50	31	2	10	26	56	8
7-1		247	145	18	52	25	5	16	36	39	9
2	3	247	136	14	51	29	6	9	34	45	12
8-1		280	144	17	55	24	5	11	38	43	8
2	3	266	110	14	52	29	5	10	37	43	10
Difference[a] Mean ± SD		19 ± 17	22 ± 21	3 ± 2	3 ± 1	4 ± 3	2 ± 2	4 ± 2	4 ± 4	4 ± 3	3 ± 3

[a]Observed differences between the two plasma samples of each individual.

larger and more buoyant HDL_{2b} and HDL_{2a} components. This shift to smaller, protein-rich particles in CAD patients provides an explanation for their low HDL_2 CH levels as well as the higher (plasma A-I + A-II)/(HDL CH) ratio detected in these patients (3.64 vs. 3.17 and 3.28 in the two control groups). Most (but not all) previous studies have demonstrated the association of low levels of HDL, in particular HDL_2 CH, with CAD. The levels of HDL_3 CH in CAD patients were either comparable to or slightly lower than controls (4, 10–12, 31, 32). Our present data are consistent with these findings. However, while the HDL_3 CH levels of CAD patients and controls were similar, it must be emphasized that the corresponding component particles were considerably smaller in CAD patients, reflecting not just quantitative but basic structural and compositional differences between the HDL of CAD patients and controls. In both HDL subpopulations, the reduction of particles in the 9.2–11.2 nm region was accompanied by a reciprocal increase of particles in the 7.0–8.2 nm region, suggesting that HDL particles in these two size regions are metabolically related. Specifically, small 7.0–8.2 nm HDL particles may be precursors of large 9.2–10.2 nm HDL particles as discussed below. We have found that preparative ultracentrifugation results in the preferential loss of very large and very small HDL particles (16). Thus, the abnormal HDL particles observed in this study may be missed and their quantification underestimated in HDL isolated by conventional ultracentrifugation techniques. Likewise, the ability of anti-A-II and anti-A-I antibodies to recognize all Lp(AI w AII) and Lp(AI w/o AII) in any given plasma or test sample is essential for the accurate isolation and quantification of these lipoproteins.

The reduction of particles corresponding to HDL_2 and the increase of small, dense HDL particles in hypertriglyceridemia have been previously described (15, 33–35). An increase of small HDL particles, however, was also observed in our CAD patients with normal TG levels. The occurrence of very large HDL particles has been shown to exist in patients with primary biliary cirrhosis (8), in cord blood (36), and in subjects with specific familial diseases such as lecithin:cholesterol acyltransferase (LCAT) deficiency, apo-A-I, C-III deficiency, fish eye disease, and abetalipoproteinemia (37–40). The metabolic and pathogenic implications of these very large and very small HDL particles are at present unknown.

The chemical composition and particle size of HDL are regulated by a host of enzymes and proteins, as well as by the presence of lower density lipoproteins that act as donors or acceptors of lipids and apolipoproteins. Lipoprotein lipase (LPL) and LCAT are involved in the conversion of small (HDL_3) particles to large (HDL_2)

particles. Hepatic lipase and lipid transfer proteins are believed to facilitate the conversion of larger to smaller particles (41). Nascent spherical Lp(AI w/o AII) isolated from HepG2-conditioned medium were mostly particles with Stokes diameters smaller than 8.2 nm (42). These small nascent HDL as well as plasma Lp(AI w AII) and Lp(AI w/o AII) in the 7.0–8.2 nm interval can be transformed to larger 9.2–11.2 nm particles in vitro by LCAT in the presence or absence of lower density lipoproteins (43, 44). The predominance of small HDL particles in CAD, therefore, reflects impaired intravascular modulation of HDL by LCAT and LPL or increased hepatic lipase or LTP-I activity. Reduction of plasma lipoprotein lipase and LCAT activity in CAD patients has been reported in several studies (45–48). Little is known with regard to the hepatic lipase and lipid transfer activity in CAD. Cholesteryl ester transfer activity measured in 10 of the 17 CAD patients in this study was within the normal to high-normal level, consistent with the relatively smaller HDL particles seen in these patients. In the Familial Atherosclerosis Treatment Study (FATS) (49), hepatic lipase was slightly correlated to the progression of atherosclerosis ($r = 0.3$, $n = 48$, $P < 0.05$). Precisely how the various enzymes and lipoproteins interact in vivo to determine the chemical composition and particle size distribution of HDL remains to be clarified. Knowledge in this direction would help delineate the metabolic defects underlying our present observations. Finally, whether these altered subpopulations are a result of the disease process or are somehow involved in atherogenesis remains to be determined. We have found similar HDL particle size abnormalities in patients with end-stage renal disease (50; J. Joven, E. Vilella, S. Ahmad, M. C. Cheung, and J. D. Brunzell, unpublished results). Impaired tissue CH removal by HDL from patients with renal failure has been reported (51). Since atherosclerotic cardiovascular disease is the major cause of death in these patients, a possible relationship between abnormal HDL particles and impaired reverse tissue CH transport may have contributed to CAD.

In conclusion, the present work represents the first report describing the size of HDL particles in CAD. It provides detailed insight into the size subspecies of Lp(AI wAII) and Lp(AI w/o AII) in HL and NL CAD patients. Our data suggest that HDL particle size distribution may be a better indicator of CAD risk than plasma total, HDL, or HDL_2 cholesterol.

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