Human apolipoprotein A-I and A-II metabolism

Ernst J. Schaefer,[1] Loren A. Zech, Leslie L. Jenkins, Thomas J. Bronzert,[2] Elizabeth A. Rubalcaba, Frank T. Lindgren, Roger L. Aamodt, and H. Bryan Brewer, Jr.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, Department of Nuclear Medicine,[3] The Clinical Center, National Institutes of Health, Bethesda, MD 20205, and Donner Laboratory,[4] Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Abstract The kinetics of the major apolipoproteins (apo) of plasma high density lipoproteins (HDL), apoA-I and apoA-II, were examined in a total of 44 individual tracer studies in 22 normal male and female subjects. Following the intravenous injection of radioiodinated HDL, the specific radioactivity decay of apoA-I within HDL (residence time, 5.07 ± 1.53 days), as determined by column chromatography, was significantly ($P < 0.01$) faster than that of apoA-II (residence time, 5.96 ± 1.84 days). The specific radioactivity decay of apoA-I within HDL when labeled on HDL or as apoA-I was found to be almost identical. Similar results were obtained for apoA-II. Analysis of simultaneous paired radiolabeled apoA-I and apoA-II studies revealed that the mean apoA-I plasma residence time (4.46 ± 1.04 days) was significantly ($P < 0.01$) shorter than that for apoA-II (4.97 ± 1.06 days). Females had significantly ($P < 0.01$) higher apoA-I plasma concentrations (124 ± 24 mg/dl) and apoA-I synthesis rates (13.58 ± 2.23 mg/kg · day) than did males (108 ± 16 mg/dl, and 11.12 ± 1.92 mg/kg · day, respectively). Plasma apoA-I levels were correlated with plasma apoA-I residence times, but not synthesis rates; and apoA-II concentrations were correlated only with apoA-II whole body residence times. ApoA-I and apoA-II plasma residence times were inversely correlated wtih plasma triglyceride levels. These data are consistent with the following concepts: *1*) labeling of apoA-I and apoA-II as apolipoproteins or on HDL does not affect their specific radioactivity decay within HDL; *2*) the mean residence time of apoA-I both in plasma and in HDL is significantly shorter than that of apoA-II; *3*) the increased apoA-I levels seen in female subjects are due to increased apoA-I synthesis; and *4*) the plasma apoA-I residence time, which is inversely correlated with plasma triglyceride levels, is an important determinant of apoA-I concentration in both males and females.—**Schaefer, E. J., L. A. Zech, L. L. Jenkins, T. J. Bronzert, E. A. Rubalcaba, F. T. Lindgren, R. L. Aamodt, and H. B. Brewer, Jr.** Human apolipoprotein A-I and A-II metabolism. *J. Lipid Res.* 1982. **23:** 850–862.

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Apolipoproteins (apo) A-I and A-II are the major protein moieties of human high density lipoproteins (HDL), comprising approximately 90% of total HDL protein mass (1, 2). HDL particles containing both apoA-I and apoA-II have been isolated, and these particles appear to be the major lipoprotein species within HDL (3, 4). In addition, an HDL subfraction containing mainly apoA-I has been reported (4). Approximately 90% of plasma apoA-I and apoA-II mass is found within HDL, with the remainder being mainly in the 1.21 g/ml infranate, and a small fraction being found in the 1.063 g/ml supernate (5–7).

ApoA-I is a single polypeptide of 243 amino acid residues, and its sequence, as reported by Brewer et al. (8), differs in several positions from earlier reports by Baker et al. (9). ApoA-I contains no carbohydrate, and has a molecular weight of approximately 28,000 (8–11). This apolipoprotein has been reported to activate lecithin:cholesterol acyltransferase, the enzyme that is responsible for cholesterol esterification in plasma (12). The other major protein within HDL, apoA-II, has a molecular weight of approximately 17,000 and consists of two identical 77-amino-acid peptide chains attached by a single disulfide bond (13). ApoA-II contains no carbohydrate. Both apoA-I and apoA-II can self-associate in aqueous solutions, and this self-association results in major changes in secondary structure (14–16). ApoA-I is much more readily dissociated from HDL particles by ultracentrifugation than is apoA-II (17). Both apoA-I and apoA-II can combine with lecithin to form protein-phospholipid complexes with a hydrated density of HDL (18).

High density lipoproteins are found in human plasma in the density range 1.063–1.21 g/ml, and consist by weight of approximately 50% protein, 25% phospholipid, 20% cholesterol, and 5% triglyceride (4, 19). The cholesterol component of HDL has been inversely associated with the incidence of coronary heart disease in man (20–23). Total HDL levels in human plasma are approximately 250 mg/dl and HDL has classically been divided into two density classes: HDL_2 (d 1.063–1.125 g/ml), and HDL_3 (d 1.125–1.21 g/ml) (24). The apoA-I:apoA-II weight ratio appears to be considerably higher in

Abbreviations: apo, apolipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; KBr, potassium bromide; SR, synthesis rate; RT, residence time.

[1] Address reprint requests to Dr. E. J. Schaefer, Building 10, Room 7N117, National Institutes of Health, Bethesda, MD 20205.

[2] Current address: Beckman Instruments, Inc. Silver Spring, MD.

[3] R. L. Aamodt.

[4] F. T. Lindgren.

HDL_2 than in HDL_3 (6). A negative correlation between HDL_2 and very low density lipoproteins (VLDL) has been noted (24). More recently HDL_2 has been further subfractionated into HDL_{2b} (d 1.063–1.10 g/ml) and HDL_{2a} (1.10–1.125 g/ml) (25). Other workers, however, contend that while there are at least three components within HDL, two of these components are in HDL_3 (d 1.125–1.21 g/ml), and have termed these HDL_{3L} and HDL_{3D} (26). Females have considerably higher HDL_2 levels than do males, and population studies suggest that fluctuations in HDL levels are largely due to changes within HDL_2 (24, 27).

Radioiodinated lipoprotein and apolipoproteins kinetic studies have been helpful in understanding lipoprotein metabolism (28). In this study we wished to investigate: *1*) whether apoA-I and apoA-II were metabolized at similar or disparate rates within HDL and plasma when labeled on HDL or as apolipoproteins; *2*) whether labeling these apolipoproteins on HDL or as apolipoproteins would affect their kinetic behavior; *3*) whether human males and females have similar or disparate synthesis rates and fractional catabolic rates for apoA-I and apoA-II; and *4*) whether apoA-I and apoA-II kinetic parameters relate to plasma levels of apoA-I, apoA-II, lipids, lipoproteins, HDL lipid constituents (cholesterol, phospholipid, and triglyceride), and HDL subfractions (HDL_{2b}, HDL_{2a}, and HDL_3). We therefore studied the kinetics in plasma and HDL of radiolabeled HDL (1.063–1.21 g/ml), apoA-I, and apoA-II in normal male and female subjects.

METHODS

Subjects studied

The age, sex, height, weight, plasma lipid, and lipoprotein cholesterol values for the subjects studied are given in **Table 1**. Mean values ± SD are given for weights, and plasma cholesterol and triglyceride concentrations. Weights were determined daily during the course of each study on a metabolic scale, and plasma lipids were measured on all blood samples obtained dur-

TABLE 1. Subjects studied[a]

Subject	Age	Height	Weight	Plasma		Cholesterol		
				Cholesterol	Triglyceride	VLDL	LDL	HDL
	yrs	*cm*	*kg*			*mg/dl*		
Males								
1	22	182	74.4 ± 0.2	184 ± 5	51 ± 4	6	122	56
2	23	184	72.3 ± 0.1	119 ± 4	42 ± 2	5	60	54
3	22	175	68.1 ± 0.2	142 ± 4	81 ± 7	14	84	52
4	21	181	67.0 ± 0.1	133 ± 6	58 ± 3	10	76	51
5	21	186	78.8 ± 0.2	148 ± 5	75 ± 6	14	92	48
6	19	175	84.1 ± 0.4	177 ± 3	80 ± 7	15	126	42
7	22	177	64.2 ± 0.2	171 ± 3	79 ± 5	16	116	39
8	25	187	78.6 ± 0.3	162 ± 8	139 ± 12	26	98	38
9	23	182	94.3 ± 0.5	221 ± 11	103 ± 14	19	171	31
10	20	175	91.7 ± 0.4	191 ± 7	108 ± 11	20	142	29
11	25	179	85.0 ± 0.4	144 ± 6	192 ± 15	53	63	28
Females								
1	21	168	59.5 ± 0.2	151 ± 5	43 ± 6	4	77	70
2	20	168	64.7 ± 0.1	177 ± 10	61 ± 5	8	100	69
3	21	172	59.7 ± 0.3	192 ± 6	79 ± 8	14	119	59
4	20	158	67.8 ± 0.3	141 ± 3	85 ± 6	16	71	58
5	21	172	50.2 ± 0.1	159 ± 4	40 ± 4	8	98	53
6	22	168	76.1 ± 0.4	208 ± 12	124 ± 11	24	139	45
7	20	171	62.4 ± 0.1	163 ± 8	71 ± 7	13	107	43
8	23	160	57.1 ± 0.2	177 ± 7	84 ± 9	15	121	41
9	21	170	62.3 ± 0.3	153 ± 8	122 ± 15	22	91	40
10	20	173	63.0 ± 0.2	159 ± 8	115 ± 10	21	100	38
11	20	168	64.7 ± 0.3	177 ± 9	136 ± 11	18	122	37
Mean values ± SD								
Males	22 ± 2	180 ± 4	78.0 ± 10.0	163 ± 30	92 ± 43	18 ± 13	105 ± 34	43 ± 10
Females	21 ± 2	168 ± 5	62.5 ± 6.5	169 ± 20	87 ± 33	15 ± 6	104 ± 20	50 ± 12[b]
All subjects	21 ± 2	174 ± 7	70.3 ± 11.4	166 ± 25	90 ± 41	16 ± 10	104 ± 35	46 ± 12

[a] Weights are given as the mean of all determinations ± SD. Plasma lipid levels are the mean ± SD of 15 fasting determinations, and lipoprotein cholesterol levels were measured at the beginning of each study by LRC methodology. Subjects are grouped from highest HDL cholesterol to the lowest level by sex.

[b] Significantly different ($P < 0.01$, standard t test analysis) from corresponding male value.

ing the course of metabolic studies. Plasma lipid and lipoprotein cholesterol values were measured by established Lipid Research Clinics methodology utilizing the Autoanalyzer II (29, 30). Mean values ± SD for normal subjects (n = 1088) as measured in our laboratory in mg/dl are: plasma cholesterol 189 ± 40, plasma triglyceride 87 ± 43, VLDL cholesterol 16 ± 11, LDL cholesterol 123 ± 35, and HDL cholesterol 50 ± 14 (31). All subjects had normal hepatic, renal, and thyroid function, were not on medication known to affect plasma lipids, and were studied while on an isocaloric, 20% protein, 40% fat (polyunsaturated fat:saturated fat ratio 0.1–0.3), 40% carbohydrate, 300 mg cholesterol diet. This diet was begun at least 2 weeks prior to the start of a metabolic study and continued throughout the course of each study. All tracer studies were of 14 days duration, except for studies in female subjects 6, 7, 9, and 11 which were 13 days in length, and studies in female subject 2 and male subjects 3, 10, and 11 which were 21 days in duration. Normal activity was permitted, and all subjects were requested to adhere to the same amount of physical activity per day. Subjects were given supersaturated potassium iodide (0.5–1.0 g/day) to block thyroid uptake of radioactivity and ferrous sulfate (900 mg/day) before and during each metabolic study. Informed consent was obtained from all subjects.

Isolation and labeling of lipoproteins and apolipoproteins

High density lipoproteins were isolated under sterile conditions from plasma by sequential ultracentrifugation (in the density range 1.063–1.21 g/ml utilizing KBr for density adjustments) in Beckman L265B ultracentrifuges and 60 Ti rotors (Beckman Instruments, Inc., Fullerton, CA.) at 59,000 rpm at 4°C (32). HDL preparations were then extensively dialyzed against 0.85% NaCl, 0.01% EDTA, 0.1 M Tris (pH 7.4) solution, and the protein content was determined by the method of Lowry et al. (33). The preparations were diluted 1:1 with sterile 1 M glycine (pH 10) buffer, and radioiodinated by the iodine monochloride method of McFarlane (34). ^{125}I or ^{131}I for radioiodination was obtained from New England Nuclear Corp. (Boston, MA). The mean protein concentration (±SD) of preparations that were iodinated was 6.1 ± 1.2 mg/ml. The amount of lipid labeling was determined after lipid extraction as previously described (19, 35, 36).

Apolipoproteins A-I and A-II were isolated from normal HDL (d 1.09–1.21 g/ml) by column chromatography as previously described (8, 13). These proteins migrated as discrete bands on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) utilizing a modification of the method of Weber and Osborn as previously described (36, 37). Apolipoproteins formed a line of immunoprecipitation only against their own respective antisera when tested with antisera for apoA-I, apoA-II, and apoB, apoC-II, apoC-III, apoE, and albumin. ApoA-I and apoA-II were iodinated at a mean protein concentration of 3.6 ± 1.1 mg/ml in sterile 1 M glycine, 0.1 M Tris (pH 8.5) buffer by the iodine monochloride method (34). Vortexing was not used in the labeling procedure. For all radiolabeled preparations the efficiency of iodination was assessed by precipitation of protein with 20% trichloroacetic acid following a 1:1 dilution with 5% bovine serum albumin solution. Unbound iodine was removed by extensive dialysis against sterile 0.85% NaCl, 0.01% EDTA, 0.1 M Tris (pH 7.4). The percentage of free iodine present in radiolabeled preparations following dialysis was assessed by precipitation of protein with 20% trichloroacetic acid following the addition of 5% bovine serum albumin, and was also measured by descending strip paper radiochromatography (85% methanol on Whatman number 1 filter paper), utilizing a Packard Model 7201 radiochromatogram scanner (Packard Instrument Co., Downers Grove, IL). Radiolabeled preparations were subjected to SDS PAGE to ascertain the distribution of radioactivity among apolipoproteins. All preparations were diluted 1;10 with sterile 5% human albumin to minimize radiation damage, subjected to Millipore filtration (0.45 micron filters, Millipore Corp.), and tested for pyrogenicity and sterility prior to use.

For single isotope studies, a mean of 88 ± 6 μCi of ^{125}I was used and for double isotope studies means of 89 ± 5 μCi of ^{125}I and 21 ± 8 μCi of ^{131}I were injected intravenously. A mean of 0.8 ± 0.2 mg of labeled HDL protein and 0.3 ± 0.1 mg of labeled apoprotein in a mean volume of 0.7 ± 0.3 ml were injected intravenously in tracer studies. Male subjects 1, 2, 5–8 and female subjects 1, 3, 4, 6, 7, 9–11 simultaneously received radioiodinated apoA-I and apoA-II. Male subjects 3 and 10 received ^{125}I-labeled HDL and ^{131}I-labeled apoA-I, and male subjects 4 and 11 received ^{125}I-labeled HDL and ^{131}I-labeled apoA-II. Male subject 9 and female subject 2 received ^{125}I-labeled apoA-I only, and female subjects 5 and 8 received ^{125}I-labeled HDL only. In addition, male subjects 2, 5–7 received ^{125}I-labeled HDL during a different time period than the paired apolipoprotein tracer study.

Measurement of radioactivity and apolipoprotein and lipoprotein levels during metabolic studies

In all metabolic studies blood was obtained in 0.1% EDTA just prior to injection of radioactivity, at 10 min, 6 hr, and 12 hr after injection, and then daily in the morning for the duration of the study. Plasma was separated from blood at 4°C in a refrigerated centrifuge. Whole body ^{131}I radioactivity was measured prior to each

study and daily, except on weekend days, during each study, using a triple detector system as previously described (38).

Plasma obtained during each study at 10 min, 6, 12, and 24 hr, and at day 4, 7, 10, and 13 or 14 after injection was subjected to sequential ultracentrifugation at densities 1.063 g/ml and 1.21 g/ml in 40.3 Beckman rotors at 39,000 rpm at 4°C for the isolation of HDL (32). The protein content in HDL was determined by the method of Lowry et al. (33), the phospholipid content by the method of Chalvardjian et al. (39), and the cholesterol and triglyceride content by Autoanalyzer II methodology (Technicon Corp., Tarrytown, NJ), following lipid extraction (35). Radioactivity in plasma, lipoprotein fractions, and SDS PAGE was assessed using a Packard Model 3375 autogamma counter.

Column chromatography of HDL

In studies performed on male subjects 3, 4, 10, and 11 and female subjects 5 and 8, HDL fractions (d 1.063–1.21 g/ml), obtained at 10 min, 6 hr, 12 hr, 24 hr, and days 4, 7, 10, and 14 after injection of radiolabeled HDL, were delipidated with chloroform–methanol 2:1, lyophilized, and subjected to Sephacryl G-200 column chromatography in 6 M urea, 0.1 M Tris, pH 8.2 buffer utilizing 200 × 2.0 cm glass columns (Kontes Glass Co.). In this manner apoA-I and apoA-II specific radioactivity could be assessed. A typical column profile is shown in **Fig. 1**. The purity of apoA-I and apoA-II column fractions was assessed by SDS PAGE, and the protein content was measured by absorbance at 280 nm as well as by the method of Lowry et al. (33).

Calculation of apolipoprotein kinetic parameters

Specific radioactivity decay curves within HDL and plasma were analyzed utilizing the SAAM simulator (40) on a VAX 11/780 computer (Digital Equipment Co., Maynard, MA). Curves required three exponentials for adequate curve fitting, and residence times (1/fractional catabolic rate) were obtained from the area under the curve (41). The fractional standard deviations of residence times obtained by curve fitting were <0.10. Apolipoprotein synthesis rates (SR) were calculated utilizing the formula:

$$SR = \frac{(\text{plasma volume}) \times (\text{plasma apolipoprotein concentration})}{\text{plasma apolipoprotein residence time}}$$

Data were subjected to standard *t* test and correlation coefficient analysis (42).

Analytic ultracentrifugation. Analytic ultracentifugation of plasma samples was performed and the computer-derived schlieren patterns were obtained by procedures (24) that corrected for the concentration dependence of flotation rate and the Johnston-Ogston effect. HDL_{2b}, HDL_{2a}, and HDL_3 concentrations were determined as previously described (27).

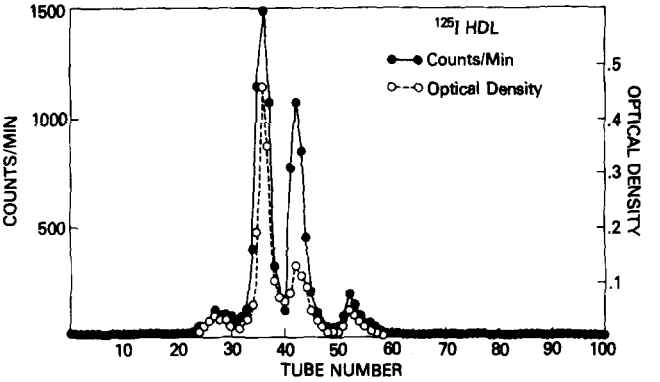

Fig. 1. Profiles of radioactivity and absorbance elution from Sephacryl G-200 column used for specific activity determination of apoA-I and apoA-II. The 2.0 × 200 cm glass column was packed with Sephacryl G-200 and eluted wth 0.1 M Tris, 6 M urea, pH 8.2 buffer. Ten to twelve mg of delipidated HDL were applied to the column and 2-ml fractions were collected.

Apolipoprotein quantitation. The concentration of apoA-I and apoA-II in all samples was measured by radial immunodiffusion (6), following delipidiation with methanol–diethyl ether 3:7, and resolubilization in 0.05 M sodium barbital, 9 mM sodium azide, pH 8.3 (barbital buffer) for immunochemical measurement (43). Recoveries of apoA-I and apoA-II pure standards following dilipidation were 98.0 ± 1.0% and 96.2 ± 0.90%, respectively. Apolipoprotein standards were obtained as previously described (8, 10, 13) and their concentrations were determined by amino acid analysis on a Beckman Model 121 Automatic Amino Acid Analyzer (Beckman Instruments, Inc.). The gamma globulin fraction was obtained by octanoic acid precipitation, and for apoA-I 3 ml and for apoA-II 8 ml of antisera in barbital buffer, respectively, were added to 1.5% agarose, 5% dextran, barbital buffer solution (100 ml), utilized for preparation of radial immunodiffusion plates. Monospecific antisera for apoA-I and apoA-II were raised in goats. Standards were utilized for each radial immunodiffusion plate. Standard curves for apoA-I were linear over a concentration range from 0.25 to 16.0 mg/dl and for apoA-II over a range from 0.5 to 10.0 mg/dl. Plasma and HDL fractions were diluted 1:15 for apoA-I determinations and 1:5 for apoA-II measurements. The mean within-run coefficients of variation for the apoA-I and apoA-II assays were 3.7% and 4.3%, respectively, and the mean between-run coefficients of variations for these assays were 4.5% and 5.9%, respectively.

Column chromatography of plasma. Gel permeation chromatography was performed with a 100 × 1.2 cm column (Kontes Glass Co.) of Sepharose 6B-CL (Pharmacia Fine Chemicals) in NaCl-EDTA buffer (0.85% NaCl, 0.01% EDTA, 1 mM sodium azide, pH 7.4) at room temperature (24°C). Plasma sample volumes applied to the column were 5 ml and flow rates were approximately 15 ml/hour. Preparations of HDL_{2b} (d 1.063–1.10 g/ml), HDL_{2a+3} (d 1.10–1.21 g/ml), and low density lipoproteins (d < 1.063 g/ml) applied to this column were isolated from a normolipemic individual by ultracentrifugation as previously described. Radiolabeled apolipoproteins (1–2 μl) were incubated with 5 ml of plasma for 30 min at 37°C prior to application on the column. In addition, in all studies, tracers were incubated as described above with plasma obtained from each subject just prior to injection, and subjected to ultracentrifugation for the isolation of HDL (d 1.063–1.21 g/ml).

RESULTS

Plasma lipid and lipoprotein data

Plasma lipid and lipoprotein cholesterol concentrations on all study subjects are given in Table 1. Subjects are grouped in this and subsequent tables from highest to lowest HDL cholesterol value by sex for purposes of comparison. Plasma lipid values on all subjects were within two standard deviations of the normal mean for our laboratory (31) and were within the normal range (5–95th percentile) of the Lipid Research Clinics population, except for female subject 11 who had a triglyceride level that was 5 mg/dl above the 95th percentile (44). Fluctuation in body weight and fasting plasma lipid levels were quite small as can be seen from the standard deviation values in Table 1. Females had significantly ($P < 0.01$) higher HDL cholesterol values, and slightly lower VLDL cholesterol and plasma triglyceride levels, than did males.

Analytical ultracentrifugation data are presented in **Table 2**. Female subjects had significantly higher total HDL, HDL_{2b}, and HPL_{2a} concentrations, and slightly lower VLDL levels than did male subjects. No significant sex differences with regard to HDL_3 and LDL levels were noted. Total HDL values were significantly ($P < 0.01$) correlated with HDL_{2b} and HDL_{2a} values, and were inversely ($P < 0.01$) correlated with VLDL levels. HDL_{2b} values were also inversely correlated ($P < 0.01$) with VLDL concentrations. No correlations with LDL and HDL_3 levels were noted.

TABLE 2. Analytical ultracentrifugation data; total lipoprotein levels in mg/dl[a]

Subjects	VLDL	IDL	LDL	HDL_{2b}	HDL_{2a}	HDL_3	HDL_{Total}
				mg/dl			
Males							
1	12	4	287	65	95	134	294
2	1	3	165	136	93	87	316
3	16	12	174	49	126	109	284
4	12	11	172	73	92	101	266
5	16	2	205	43	83	114	240
6	11	12	288	26	70	113	209
7	35	22	265	24	81	112	217
8	91	14	231	8	42	131	181
9	87	23	315	9	52	124	185
10	75	17	295	7	51	152	210
11	281	32	150	6	30	143	179
Females							
1	5	20	164	137	93	101	331
2	15	6	216	157	131	90	378
3	16	11	265	79	172	114	365
4	22	27	151	50	161	99	310
5	12	14	214	80	107	135	322
6	18	40	324	31	114	112	257
7	38	9	239	59	81	97	237
8	47	54	266	33	130	98	261
9	67	43	239	22	107	138	267
10	38	2	245	9	64	126	199
11	40	3	268	18	72	134	224
Mean values ± SD							
Males	58 ± 81	14 ± 9	232 ± 60	41 ± 40	74 ± 28	120 ± 19	235 ± 49
Females	29 ± 19	21 ± 18	235 ± 65	61 ± 48[b]	112 ± 34[c]	113 ± 18	286 ± 59[b]
All subjects	43 ± 59	17 ± 14	234 ± 61	51 ± 45	93 ± 36	117 ± 18	261 ± 59

[a] Represents the analytical ultracentrifugation lipoprotein values on plasma samples obtained just prior to injection. The flotation rate (S_f, Svedbergs) of lipoproteins is VLDL ($S_f > 20$), IDL (S_f 12–20), LDL (S_f 0–12), and HDL (HDL flotation rate, F° 0–9) (24, 26).

[b] Significantly ($P < 0.05$) different from male values.

[c] Significantly ($P < 0.01$) different from male values.

Compositional data on HDL (d 1.063–1.21 g/ml) in study subjects are shown in **Table 3**. Female subjects had significantly higher HDL protein, phospholipid, and cholesterol concentrations, and slightly lower HDL triglyceride levels, than did males. Male subjects with the lowest levels of total HDL, tended to have a higher fraction of HDL as protein and triglyceride, and a lower fraction of HDL as phospholipid and cholesterol; the converse was true for males with the highest levels of HDL. These trends were not as striking in female subjects.

Characterization of radioiodinated HDL and apolipoproteins

The mean efficiency of iodination of HDL preparations was 55.1 ± 5.0%, and the mean lipid labeling was 1.9 ± 0.3%. Assuming a molecular weight of 30,000 for HDL protein, a mean of 0.6 ± 0.1 moles of iodine per mole of HDL protein was incorporated into HDL during the labeling procedure. All radiolabeled HDL preparations utilized for tracer studies were shown to contain no free iodine by radiochromatography, less than 1% free iodine by TCA precipitation, and were sterile and nonpyrogenic. All preparations were autologous, and when incubated with autologous plasma (30 min, 37°C), a mean of 2.2 ± 1.0% of radioactivity was associated with the d 1.063 g/ml supernate, 91.7 ± 2.3% with HDL, and 6.1 ± 1.4% with the d 1.21 g/ml infranate. Following injection of radioiodinated HDL into study patients (at the 10-min point), a mean of 2.1 ± 0.9% of radioactivity was associated with the d 1.063 g/ml supernate, 91.4 ± 2.1% with HDL, and 6.5 ± 0.4% with the d 1.21 g/ml infranate. No significant change in the distribution of radioactivity among lipoprotein fractions was noted throughout the course of any study. When HDL preparations were subjected to SDS PAGE, a mean of 46.1 ± 3.4% of radioactivity was associated with the apoA-I band, 39.4 ± 5.6% with apoA-II, 11.1 ± 2.2% with high molecular weight constituents, and 3.4 ± 1.7% with the C apolipoproteins.

The mean efficiencies of iodination of apoA-I and apoA-II preparations were 52.4 ± 6.9% and 62.2 ± 9.1%, respectively. Assuming a molecular weight of 28,000 for apoA-I and 18,000 for apoA-II, a mean of 0.6 ± 0.2 moles of iodine per mole of protein was incorporated into apoA-I, and for apoA-II, 0.9 ± 0.1. All radioiodinated

TABLE 3. High density lipoprotein composition[a]

Subjects	Protein	Phospholipid	Cholesterol	Triglyceride
		mg/dl		
Males				
1	142 ± 6 (0.49)	85 ± 8 (0.29)	54 ± 2 (0.19)	9 ± 2 (0.03)
2	152 ± 8 (0.50)	87 ± 5 (0.28)	55 ± 4 (0.18)	12 ± 3 (0.04)
3	135 ± 2 (0.48)	85 ± 7 (0.30)	50 ± 4 (0.18)	12 ± 2 (0.04)
4	136 ± 4 (0.49)	79 ± 10 (0.29)	49 ± 3 (0.18)	11 ± 3 (0.04)
5	129 ± 3 (0.50)	70 ± 8 (0.27)	46 ± 4 (0.18)	12 ± 1 (0.05)
6	122 ± 2 (0.53)	59 ± 5 (0.26)	38 ± 4 (0.17)	11 ± 2 (0.05)
7	121 ± 5 (0.53)	58 ± 3 (0.25)	38 ± 3 (0.17)	13 ± 2 (0.06)
8	115 ± 4 (0.54)	49 ± 4 (0.23)	35 ± 6 (0.16)	14 ± 3 (0.07)
9	105 ± 3 (0.57)	37 ± 2 (0.20)	30 ± 3 (0.16)	12 ± 1 (0.07)
10	111 ± 4 (0.57)	42 ± 5 (0.22)	30 ± 4 (0.15)	12 ± 2 (0.06)
11	114 ± 4 (0.62)	34 ± 3 (0.18)	24 ± 5 (0.13)	13 ± 1 (0.07)
Females				
1	181 ± 13 (0.51)	91 ± 7 (0.26)	74 ± 6 (0.21)	10 ± 1 (0.03)
2	155 ± 11 (0.48)	90 ± 9 (0.28)	68 ± 7 (0.21)	9 ± 2 (0.03)
3	157 ± 7 (0.49)	93 ± 10 (0.29)	60 ± 6 (0.19)	10 ± 3 (0.03)
4	151 ± 6 (0.49)	91 ± 8 (0.29)	56 ± 5 (0.18)	11 ± 1 (0.04)
5	156 ± 6 (0.51)	87 ± 3 (0.29)	53 ± 2 (0.17)	9 ± 2 (0.03)
6	154 ± 11 (0.53)	72 ± 7 (0.25)	47 ± 1 (0.16)	15 ± 2 (0.05)
7	135 ± 9 (0.54)	64 ± 3 (0.25)	42 ± 5 (0.17)	10 ± 1 (0.04)
8	142 ± 6 (0.55)	65 ± 2 (0.25)	41 ± 4 (0.16)	12 ± 3 (0.05)
9	121 ± 8 (0.50)	77 ± 2 (0.32)	36 ± 3 (0.15)	9 ± 2 (0.04)
10	107 ± 10 (0.53)	52 ± 3 (0.26)	34 ± 5 (0.17)	10 ± 3 (0.05)
11	112 ± 11 (0.55)	49 ± 3 (0.24)	33 ± 2 (0.16)	10 ± 2 (0.05)
Mean values ± SD				
Males	126 ± 14 (0.52)	62 ± 20 (0.26)	41 ± 11 (0.17)	12 ± 1 (0.05)
Females	143 ± 22 (0.51)[c]	76 ± 16 (0.27)[c]	49 ± 14 (0.18)[c]	10 ± 2 (0.04)[b]
All subjects	134 ± 20 (0.52)	69 ± 19 (0.27)	45 ± 13 (0.17)	11 ± 2 (0.04)

[a] Represents the mean ± SD of HDL (d1.063–1.21 g/ml) compositional data obtained on six fasting samples (10 min; days 1, 4, 7, 10, and 13 or 14) from each metabolic study. Values in parentheses indicate the fraction of total HDL represented by each constituent.
[b] Significantly different ($P < 0.05$, standard t test analysis) from comparable male values.
[c] Significantly different ($P < 0.01$, standard t test analysis) from comparable male values.

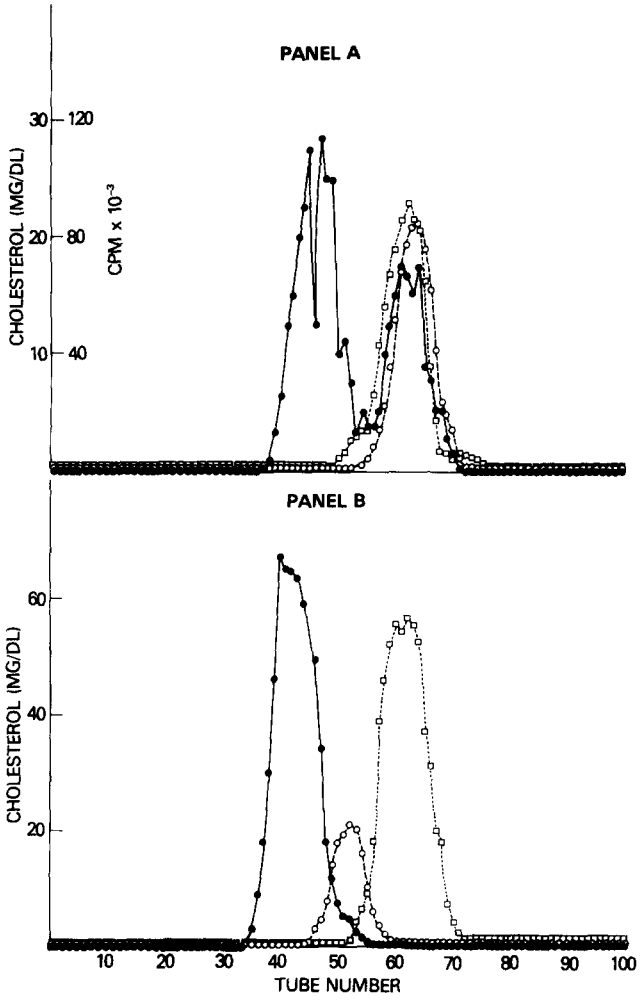

Fig. 2. A. Column elution profile of ^{125}I-labeled apoA-I radioactivity, (□ --- □), ^{131}I-labeled apoA-II radioactivity (○ —— ○), and cholesterol (● —— ●) following the incubation (30 min, 37°C) of tracers with 5 ml of plasma. Column chromatography was performed wth a 100 × 1.2 cm glass column packed wtih Sepharose 6B-CL and eluted with 0.85% NaCl, 0.01% EDTA, 1 mM sodium azide, pH 7.4 buffer. Five ml of the incubation mixture were applied to the column, and 1.3-ml fractions were collected. B. Column elution profile on same column of d 1.063 g/ml supernate (● —— ●), HDL_{2b} (d 1.063–1.10 g/ml, ○ —— ○), and HDL_{2a+3} (d 1.10–1.21 g/ml, □ --- □).

apolipoprotein preparations contained no free iodine by radiochromatography, and less than 1% by TCA preparation; they were sterile and pyrogen-free. Means of 91.5 ± 4.1% of apoA-I radioactivity and 95.2 ± 1.2% of apoA-II radioactivity were recovered with the appropriate band on SDS PAGE. When incubated with the plasma (30 min, 37°C) of study subjects, means of 2.2 ± 1.3% of apoA-I radioactivity and 0.4 ± 0.2% of apoA-II radioactivity, respectively, were associated with the d 1.063 g/ml supernate, 90.3 ± 3.5% of apoA-I radioactivity and 96.5 ± 4.1% of apoA-II radioactivity with HDL, and 7.5 ± 1.7% of apoA-I radioactivity and 3.1 ± 0.9% of apoA-II radioactivity with the d 1.21 g/ml infranate. When apoA-I and apoA-II radiolabeled preparations were preincubated with HDL, separated by ultracentrifugation at d 1.21 g/ml, dialyzed, and then incubated with plasma, nearly identical distributions of radioactivity among lipoprotein fractions were noted. The percentages of apoA-I radioactivity in the d 1.063 g/ml supernate, HDL, and the d 1.21 g/ml infranate were 2.1 ± 0.9%, 89.8 ± 2.4%, and 8.1 ± 0.4%, respectively; and for apoA-II, 0.3 ± 0.1%, 96.8 ± 3.2, and 2.9 ± 0.2%, respectively. Following injection of radioiodinated apoA-I and apoA-II into study subjects (at the 10-min point), means of 2.7 ± 0.9% of apoA-I and 0.6 ± 0.3% of the apoA-II radioactivity were associated with the d 1.063 g/ml infranate, 90.3 ± 4.1% of apoA-I and 95.9 ± 3.6% of apoA-II radioactivity with HDL, and 7.0 ± 1.6% of apoA-I and 3.5 ± 0.9% of apoA-II radioactivity with the d 1.21 g/ml infranate. No significant change in the distribution of radioactivity among lipoprotein fractions was noted throughout the course of any study.

When radiolabeled apoA-I and apoA-II were incubated with normal plasma (30 min, 37°C), and then subjected to Sepharose 6B column chromatography, the radioactivity profile of apoA-I was somewhat different from that of apoA-II, as shown in **Fig. 2.** There was a region on the column profile, which corresponded to the HDL_{2b} region, which contained mainly apoA-I radioactivity, and almost no apoA-II radioactivity. In addition, the peak of apoA-I radioactivity eluted slightly earlier than that of apoA-II, and there was a small apoA-I radioactivity peak in the lipoprotein-free region which was not present for apoA-II.

Apolipoprotein kinetics

Male subjects 2, 5, 6, and 7 simultaneously received radiolabeled apoA-I and apoA-II, and subsequently, during a second study, while on the same diet, received autologous radioiodinated HDL. The plasma specific radioactivity decay curves for HDL, apoA-I, and apoA-II were analyzed, and the residence times are presented in **Table 4.** In all four subjects apoA-I residence times were less than those of apoA-II. The mean HDL residence time was intermediate between that of apoA-I and that of apoA-II. Male subjects 3, 4, 10, and 11 and female subjects 5 and 8 received ^{125}I-labeled HDL. In these subjects the apoA-I and apoA-II specific activity decay curves within HDL were analyzed. The mean residence time for apoA-I decay as shown in **Table 5** was significantly less than that for apoA-II.

Male subjects 3 and 10, in addition to receiving ^{125}I-labeled HDL, simultaneously received ^{131}I-labeled apoA-II. As shown in Table 5 and **Fig. 3,** the residence times of apoA-II specific radioactivity within HDL were very similar whether labeled as HDL or as apoA-II. Male subjects 4 and 11, in addition to receiving ^{125}I-labeled

TABLE 4. Plasma residence time (days) of apoA-I and apoA-II specific radioactivity labeled as HDL or as apolipoprotein

	^{125}I-labeled HDL	I-labeled Apolipoprotein[a]	
		ApoA-I	ApoA-II
Subject[b]			
2	6.71	6.16	6.35
5	6.63	5.66	7.26
6	5.87	4.62	5.70
7	4.96	4.95	5.19
Mean ± SD	6.04 ± 0.81	5.35 ± 0.069	6.13 ± 0.89

[a] Labeled either with ^{125}I or ^{131}I.
[b] All subjects were males, and ^{125}I-labeled HDL was not given simultaneously with radiolabeled apolipoproteins.

HDL, simultaneously received ^{131}I-labeled apoA-I. As shown in Table 5 and Fig. 3, as for apoA-II, the residence times of apoA-I specific radioactivity decay within HDL were very similar whether labeled as HDL or as apoA-I.

Apolipoprotein kinetic studies were performed in 11 normal male and 9 normal female subjects. Plasma concentrations, synthesis rates, and plasma and whole body residence times of apoA-I and apoA-II for study subjects are shown in **Table 6.** In 14 subjects, radiolabeled apoA-I and apoA-II were simultaneously injected. In these paired studies the mean plasma residence time for apoA-I (4.46 ± 1.04 days) was significantly ($P < 0.01$) less than that for apoA-II (4.97 ± 1.06 days). These differences were present in both males and females as can be seen in Table 6. Females had significantly higher apoA-I plasma concentrations and apoA-I synthesis rates than did males, with no significant sex differences with regard to apoA-I plasma residence time. Plasma apoA-II levels were only slightly higher in females than in males, but females had significantly higher apoA-II synthesis rates and lower apoA-II plasma residence times than did males.

Correlation coefficient analysis

ApoA-I and apoA-II plasma concentrations, synthesis rates, and plasma residence times were utilized for standard correlation coefficient analysis with other study parameters as shown in **Table 7** (42). Plasma apoA-I concentrations were significantly correlated with apoA-I plasma residence times, apoA-I whole body residence times, apoA-II whole body residence times, total HDL, HDL_{2b}, HDL cholesterol, HDL protein, and HDL phospholipid values, and inversely correlated with VLDL cholesterol and plasma triglyceride levels. No correlation with apoA-I synthesis rates was observed. Plasma apoA-II concentrations were correlated only with apoA-II whole body residence times. ApoA-I synthesis rates were highly correlated with apoA-II synthesis rates; but both

TABLE 5. Residence time (days) of apoA-I and apoA-II specific radioactivity within HDL[a]

	^{125}I-labeled HDL		^{131}I-labeled Apolipoprotein	
Subject	ApoA-I	ApoA-II	ApoA-I	ApoA-II
Males				
3	4.89	5.12		5.12
4	6.84	7.36	6.50	
10	3.50	4.33		4.39
11	3.72	4.78	3.74	
Females				
5	7.04	9.02		
8	4.44	5.03		
Mean ± SD	5.07 ± 1.53	5.94 ± 1.84[b]		

[a] Male subjects 3 and 10 simultaneously received ^{125}I-labeled HDL and ^{131}I-labeled apoA-I; male subjects 4 and 11 received ^{125}I-labeled HDL and ^{131}I-labeled apoA-II; and female subjects 5 and 8 received ^{125}I-labeled HDL. Specific radioactivity decay within HDL (d 1.063–1.21 g/ml) was determined utilizing Sephacryl G-200 column chromatography.
[b] Significantly ($P < 0.01$) different from apoA-I residence time.

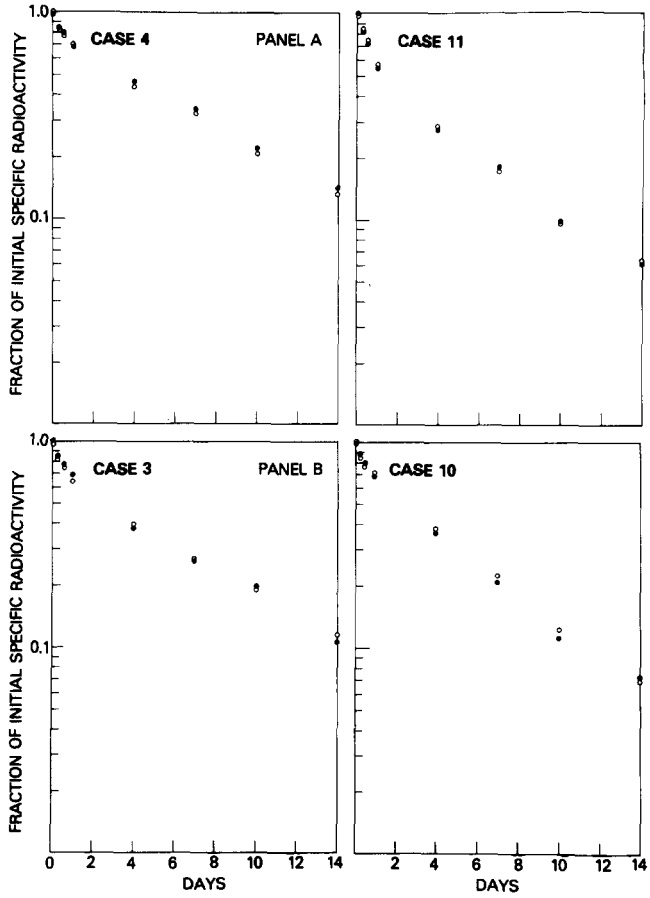

Fig. 3. A. Specific radioactivity activity decay of ^{125}I-labeled apoA-I (● —— ●) and ^{131}I-labeled apoA-I (○ —— ○) within HDL and ^{131}I-labeled apoA-I in male subjects 4 and 11. B. Specific radioactivity decay of ^{125}I-labeled apoA-II (● —— ●) and ^{131}I-labeled apoA-II (○ —— ○) within HDL following the intravenous injection of ^{125}I-labeled HDL and ^{131}I-labeled apoA-I in male subjects 3 and 10.

TABLE 6. Apolipoprotein metabolic studies

Subjects Studied	Plasma Volume	Plasma Concentrations		Synthesis Rates		Residence Times: Plasma		Residence Times: Whole Body	
		ApoA-I	ApoA-II	ApoA-I	ApoA-II	ApoA-I	ApoA-II	ApoA-I	ApoA-II
	liters	*mg/dl*		*mg/kg · days*		*days*		*days*	
Males									
1	3.37	134 ± 15	22 ± 3	12.88	2.06	4.68	4.70		7.79
2	3.24	131 ± 5	25 ± 1	9.57	1.78	6.16	6.35		11.61
3	3.07	111 ± 3	26 ± 2		2.35		4.96		8.90
4	3.02	124 ± 6	16 ± 1	11.62		4.79		9.00	
5	3.56	105 ± 4	26 ± 1	8.37	1.64	5.66	7.26	8.56	
6	3.67	97 ± 3	25 ± 3	9.45	1.98	4.62	5.70	8.09	
7	2.90	107 ± 5	23 ± 2	9.71	1.99	4.95	5.19		9.01
8	3.54	95 ± 13	21 ± 3	13.39	2.49	3.18	3.92		7.02
9	4.22	83 ± 4	24 ± 2	11.51		3.23			
10	4.13	105 ± 8	25 ± 2		2.48		4.55		8.08
11	3.85	100 ± 6	23 ± 2	13.60		3.30		6.72	
Females									
1	2.70	180 ± 20	28 ± 3	14.21	2.46	5.69	5.03		10.26
2	2.92	133 ± 7	27 ± 2	13.62		4.39			
3	2.65	119 ± 13	21 ± 2	12.71	2.08	4.20	4.46		7.24
4	3.05	125 ± 5	23 ± 2	13.93	2.35	4.04	4.44		7.79
6	3.44	131 ± 10	28 ± 3	10.70	2.10	5.51	6.03	8.29	
7	2.81	107 ± 8	23 ± 3	12.11	2.11	3.96	4.91	8.08	
9	2.82	93 ± 11	27 ± 2	12.87	2.84	3.26	4.33	6.15	
10	2.85	110 ± 13	26 ± 3	13.24	3.11	3.74	3.71		7.90
11	3.45	117 ± 20	26 ± 2	18.83	3.32	2.80	3.54	6.70	
Mean Values ± SD									
Males									
All studies (N = 11)		108 ± 16	23 ± 3	11.12 ± 1.92	2.10 ± 0.32	4.51 ± 1.07	5.33 ± 1.07	8.09 ± 0.99	8.86 ± 2.01
Paired studies (N = 6)		111 ± 17	24 ± 2	10.56 ± 2.10	1.99 ± 0.29	4.88 ± 1.02	5.52 ± 1.19[a]		
Females									
All studies (N = 9)		124 ± 24[b]	25 ± 3	13.58 ± 2.23[b]	2.52 ± 0.46[b]	4.18 ± 0.94	4.56 ± 0.79[b]	7.49 ± 1.26	8.30 ± 1.34
Paired studies (N = 8)		124 ± 26	25 ± 2	13.57 ± 2.39	2.52 ± 0.46	4.15 ± 1.00	4.56 ± 0.79[a]		
All studies (N = 20)		115 ± 21	24 ± 3	12.35 ± 2.38	2.32 ± 0.46	4.34 ± 0.99	4.94 ± 0.99	7.79 ± 1.10	8.56 ± 1.43

Paired studies are those in which radioiodinated apoA-I and apoA-II were simultaneously injected. Plasma apolipoprotein concentrations are given as the mean ± SD of all determinations. Plasma residence times were derived from radioactivity decay curves, and synthesis rates were calculated from the plasma masses and the residence times.

[a] Significantly different ($P < 0.05$, paired t test analysis) from apoA-I residence time.

[b] Significantly different ($P < 0.01$, standard t test analysis) from corresponding male values.

these parameters were not correlated with other variables. ApoA-I residence times were significantly correlated with apoA-I plasma concentrations, apoA-I whole body residence times, apoA-II plasma residence times, apoA-II whole body residence times, total HDL, HDL_{2b}, HDL cholesterol, HDL protein, and HDL phospholipid values, and inversely correlated with VLDL cholesterol and plasma triglyceride levels. ApoA-II plasma residence times were correlated with apoA-I plasma residence times, apoA-I whole body residence times, apoA-II whole body residence times, and inversely correlated with plasma triglyceride concentrations.

DISCUSSION

The HDL compositional data presented are consistent with previous observations that female subjects have significantly higher HDL levels than do males, and that fluctuations in total HDL concentrations are largely due to alterations in HDL_{2b} and HDL_{2a} (24, 27). The latter findings are due to the greater relative fluctuation in the phospholipid and cholesterol than in HDL protein in subjects with varying HDL levels (19).

The characteristics of radioiodinated HDL and its radioactivity distribution among lipoprotein fractions in

TABLE 7. Correlation coefficient analysis (r values)

	Concentration		Synthesis Rates		Residence Times	
	ApoA-I	ApoA-II	ApoA-I	ApoA-II	ApoA-I	ApoA-II
ApoA-I concentration		0.193	0.215	−0.068	0.592[a]	0.116
ApoA-I S.R.	0.215	0.065		0.834[a]	−0.068	−0.024
ApoA-I plasma R.T.	0.592[a]	0.161	−0.028	−0.049		0.851[a]
ApoA-I whole body R.T.	0.506[b]	−0.140	−0.055	−0.097	0.853[a]	0.835[a]
ApoA-II concentration	0.193		0.065	0.257	0.161	0.276
ApoA-II S.R.	−0.068	0.257	0.834[a]		−0.049	−0.228
ApoA-II plasma R.T.	0.116	0.276	−0.024	−0.228	0.851[a]	
ApoA-II whole body R.T.	0.539[b]	0.631[a]	−0.031	−0.102	0.927[a]	0.880[a]
Total HDL	0.687[a]	0.101	0.081	−0.082	0.447[b]	0.158
HDL_{2b}	0.752[a]	0.099	0.220	−0.108	0.593[a]	0.371
HDL_{2a}	0.413	0.036	0.027	−0.011	0.284	0.062
HDL_3	−0.179	0.009	0.335	0.124	−0.144	−0.118
HDL cholesterol	0.826[a]	0.103	−0.007	−0.187	0.616[a]	0.286
HDL protein	0.854[a]	0.261	−0.042	−0.121	0.663[a]	0.345
HDL phospholipid	0.660[a]	0.034	−0.084	−0.083	0.828[a]	0.278
HDL triglyceride	−0.255	−0.046	−0.391	−0.141	0.170	0.384
Plasma cholesterol	−0.188	0.230	−0.011	−0.007	−0.109	−0.034
VLDL cholesterol	−0.524[b]	−0.028	0.155	0.432	−0.540[a]	−0.355
LDL cholesterol	−0.197	0.221	−0.188	−0.196	−0.055	0.045
Plasma triglyceride	−0.512[b]	0.051	0.239	0.144	−0.652[a]	−0.502[b]

[a] Significant correlation ($P < 0.01$).
[b] Significant correlation ($P < 0.05$).
S.R., synthesis rate; R.T., residence time.

plasma either in vitro or in vivo in our experiments was similar to that reported by other investigators (36, 45–48). Initial studies of HDL apoprotein plasma kinetics documented half-lives of 3.8–4.6 days in normal subjects (45–47). More recently, Blum and coworkers (36) studied radiolabeled HDL kinetics in normal subjects, and analyzed apoA-I and apoA-II specific radoactivity decay curves within HDL by column chromatography. These investigators noted very similar terminal slopes of apoA-I and apoA-II radioactivity decay, and concluded that the fractional catabolic rates of these apolipoproteins were identical (36). Fidge et al. (48) have reported that the fractional catabolic rate of apoA-I within HDL was slightly greater than that of apoA-II following the injection of ^{125}I-labeled HDL into 4 normal and 16 hyperlipidemic subjects, but these differences were not statistically significant.

In our own studies we observed that apoA-I specific activity decay within HDL was significantly faster than that of apoA-II following the injection of ^{125}I-labeled HDL into six normal subjects (Table 5). Because of these findings we analyzed the specific radioactivity decay plots of apoA-I and apoA-II within HDL in eight normal subjects on a balanced diet as previously published by Blum et al. (36). Instead of analyzing only the terminal slopes of the decay curves, we fit the entire curve for each apoprotein in each study (utilizing two exponentials). The mean apoA-I residence time (4.24 ± 0.51 days) was significantly ($P < 0.01$) less than that of apoA-II (4.83 ± 0.75 days) in these studies. Our analysis of Blum's data, as well as our own findings, would indicate that apoA-I fractional catabolism within HDL is significantly greater than that of apoA-II.

We utilized radiolabeled apoA-I and apoA-II to fully examine the plasma kinetics of these apolipoproteins. The distribution of radioactivity of these tracers among plasma lipoprotein fractions following in vitro incubation was similar to that observed in vivo. Preincubation of radioiodinated apolipoproteins with HDL did not appear to affect this distribution. For this reason we chose to inject labeled apolipoproteins intravenously and allow them to associate with native apoA-I and apoA-II containing lipoprotein particles.

A slightly greater percentage of apoA-I radioactivity was consistently found in the d 1.063 g/ml supernate and the d 1.21 g/ml infranate than that observed for apoA-II radioactivity. In addition, as can be seen in Fig. 2, within the HDL_{2b} region on Sepharose 6B column chromatography, there was considerable apoA-I radioactivity with almost no apoA-II radioactivity. The apoA-I radioactivity peak also eluted at a slightly earlier position (Fig. 2). Kostner et al. (4) have reported that there are lipoprotein fractions within the HDL_{2b} density region that contain mainly apoA-I with very little apoA-II. A number of investigators have reported a significantly higher apoA-I:apoA-II ratio within HDL_2 than HDL_3 (4–6, 19). These observations are consistent with the concept that the distribution of apoA-I among lipoproteins is somewhat different than that of apoA-II.

In initial studies, as shown in Table 4, we observed

that HDL specific radioactivity decay rates in plasma were intermediate between apoA-I and apoA-II decay rates. In further studies, we compared ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-I specific radioactivity decay within HDL following the injection of ^{125}I-labeled HDL and ^{131}I-labeled apoA-I. The data, as shown in Fig. 3 and Table 5, indicate that labeling apoA-I as an apolipoprotein or an HDL does not appear to affect its specific activity decay within HDL. Similar results were obtained for apoA-II (Fig. 3, Table 5), and these latter findings are in agreement with observations by Shepherd et al. (49). However, these investigators noted a significant difference in apoA-I specific activity (in d 1.21 g/ml supernate following tetramethylurea precipitation) if apoA-I was labeled on HDL or as apoprotein with subsequent intercalation into HDL by incubation (in two normal subjects) (49). In a further experiment, these workers demonstrated that this difference did not appear to be due to actual labeling of apoA-I as an apolipoprotein or on HDL, but rather may have been a function of the intercalation process (49). In our experiments we did not see this difference, possibly because we allowed radiolabeled apolipoproteins to associate with an entire plasma volume of native apoA-I and apoA-II containing lipoproteins in vivo.

The results of our apolipoprotein kinetic studies are as shown in Table 6. In paired, radiolabeled apolipoprotein studies in 14 normal subjects, the mean plasma residence time of apoA-I was significantly shorter than that of apoA-II in both male and female subjects. These findings, in conjunction with the radiolabeled HDL data, support the conclusion that the catabolism of apoA-I is different from that of apoA-II whether examined in plasma or in HDL with either radiolabeled HDL or apolipoproteins.

Significant sex differences were also noted in these studies. As shown in Table 6, females had significantly higher apoA-I and slightly higher apoA-II plasma concentrations than did males, consistent with previous observations (5–7). These increased levels were due to significantly increased apoA-I and apoA-II synthesis rates in female subjects as compared to males. These data are at variance with those of Shepherd et al. (50) who noted no significant differences in apoA-I and apoA-II kinetic parameters between sexes. The increased synthesis rates of apoA-I and apoA-II that we observed may be due to hormonal influences. Estrogen administration has been shown to increase HDL protein synthesis (51), and the sex difference with regard to HDL does not appear until after the age of puberty (44).

While synthesis rates appeared to be important in determining the increased apoA-I and apoA-II concentrations seen in females, the fractional catabolic rate appeared to be a crucial determinant of apoA-I levels for the entire sample. As shown in Table 7, apoA-I plasma concentrations were significantly correlated with apoA-I plasma and whole body residence times, but not with apoA-I synthesis rates. ApoA-II levels were significantly correlated only with apoA-II whole body residence time. Both apoA-I and apoA-II synthesis rates were highly correlated with each other, as were apoA-I and apoA-II residence times (see Table 7), suggesting that the regulation of synthesis and catabolism of these apolipoproteins may be coupled, despite differences in their fractional catabolic rate.

ApoA-I residence times were positively correlated with HDL protein, phospholipid, and cholesterol concentrations, and with HDL_{2b} levels, but were negatively correlated with VLDL cholesterol and plasma triglyceride values. ApoA-II residence times were also inversely correlated with plasma triglyceride levels. An inverse association between triglyceride and HDL concentrations has long been noted (24, 31, 52–54). Furman et al. (46), and more recently Fidge et al. (48), have reported enhanced fractional catabolic rates of HDL in hypertriglyceridemic subjects. In other patients with decreased HDL levels (Tangier disease, alcoholic hepatitis), enhanced HDL apoprotein fractional catabolism has also been reported, underscoring the importance of the catabolic rate as a determinant of plasma apoA-I and apoA-II concentration (55, 56).

ApoA-I- and apoA-II-containing lipoproteins appear to enter plasma either on chylomicrons and HDL from the intestine, or on HDL from the liver (57–65). Following the entry of chylomicrons into plasma, almost all of their apoA-I and apoA-II is rapidly transferred to HDL (60, 61). In subjects with increased plasma triglyceride-rich lipoproteins, the fractional catabolism of apolipoproteins A-I and A-II appears to be enhanced (46, 48, 55).

The data presented are consistent with the following concepts: *1*) labeling of apoA-I and apoA-II as apolipoproteins or on HDL does not affect their specific activity decay within HDL; *2*) apoA-I is catabolized at a higher fractional rate than apoA-II in both plasma and HDL; *3*) females have significantly higher apoA-I and apoA-II synthesis rates than do males; and *4*) apoA-I fractional catabolic rate, which is correlated with plasma triglyceride, is an important determinant of apoA-I plasma concentration.

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