Metabolism of high density lipoprotein subfractions and constituents in Tangier disease following the infusion of high density lipoproteins[1]

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Abstract The metabolism of apolipoproteins A-I and A-II, as well as other high density lipoprotein (HDL) constituents, was studied in patients with homozygous familial HDL deficiency (Tangier disease) prior to and after plasma exchange or HDL infusion. Mean plasma apoA-I, apoA-II, and HDL cholesterol values in homozygotes (n = 2) were 2.0 mg/dl, 2.7 mg/dl, and 1.5 mg/dl, respectively, and in a normal control subject were 125.1 mg/dl, 23.0 mg/dl, and 53.0 mg/dl, respectively. Based on radioiodinated apoA-I and apoA-II kinetic studies in the baseline state, synthesis rates for apoA-I and apoA-II in mg/kg/day were 3.81 and 1.61, respectively, in one homozygote (patient B) and 11.82 and 1.99, respectively, in the normal subject. ApoA-I and apoA-II plasma residence times in days were 0.22 and 0.81, respectively, in the homozygote, and 4.04 and 4.44, respectively, in the normal subject. These data indicate that this homozygote had both a moderate decrease in the synthetic rates of apoA-I and apoA-II, as well as a marked decrease in the plasma residence times of these two apolipoproteins. In one homozygote (patient A) following a complete plasma exchange during cardiopulmonary bypass, plasma HDL cholesterol, apoA-I, and apoA-II levels were very similar to pre-exchange values within 64 hr after exchange. A second homozygote (patient B) received HDL intravenously as well as ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II. Following infusion, the residence time in days for HDL subfractions, HDL_{2b}, HDL_{2a}, and HDL_3 were 0.1, 0.8, and 2.7, respectively. HDL protein and phospholipid both had a monoexponential decay, with residence times of 0.7 days, while HDL triglyceride disappeared monoexponentially with a residence time of 0.5 days. HDL cholesterol had a biexponential decay, with the residence time of the slow component being 0.7 days. Plasma and HDL apoA-I decayed down to baseline values significantly faster than did plasma and HDL apoA-II. ApoA-II specific radioactivity decreased throughout the course of the infusion study in both plasma and HDL, while apoA-I specific radioactivity decreased slightly, then rose, and subsequently declined in both plasma and HDL. The data indicate that the rapid and altered catabolism of apoA-I and apoA-II in Tangier homozygotes persists despite major increases in the plasma pool size of these proteins. In addition, following HDL infusion, HDL_{2b} and HDL_{2a} disappeared at a faster rate than HDL_3, HDL cholesterol and triglyceride were catabolized at a faster rate than HDL protein and phospholipid, and apoA-I disappeared more rapidly than apoA-II. These observations may have important implications with regard to the catabolism of HDL subfractions and constituents in normal man.—**Schaefer, E. J., D. W. Anderson, L. A. Zech, F. T. Lindgren, T. B. Bronzert, E. A. Rubalcaba, and H. B. Brewer, Jr.** Metabolism of high density lipoprotein subfractions and constituents in Tangier disease following the infusion of high density lipoproteins. *J. Lipid Res.* 1981. **22:** 217–228.

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High density lipoproteins (HDL), as conventionally isolated from normal human plasma in the density range 1.063–1.21 g/ml, are a polydisperse collection of particles composed of phospholipid, free and esterified cholesterol, triglyceride, and various proteins (1–4). Apolipoproteins (apo) A-I, A-II, B, C-I, C-II, C-III, D, E, and F are all protein constituents of HDL (2–6). ApoA-I and apoA-II comprise approximately 90% of HDL protein mass, with an apoA-I: apoA-II weight ratio ranging from about 6:1 to 2:1 (1, 2, 4). ApoA-I is a single polypeptide consisting of 243 amino acid residues (7–9). ApoA-I has been reported to activate the enzyme lecithin cholesterol acyl transferase (LCAT), which is responsible for plasma cholesterol esterification (10). ApoA-II in man is a single protein consisting of two identical peptides of 77 residues each, linked by a single disulfide bond (11, 12).

HDL particles containing both apoA-I and apoA-II have been isolated (2). In addition, Kostner et al. (1)

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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have reported the presence of an HDL subfraction containing only apoA-I, while Assmann et al. (13) have isolated HDL particles from patients with Tangier disease containing only apoA-II. The presence of distinct lipoprotein "families" within HDL containing the A, B, C, D, and E apolipoproteins, respectively, has been reported by Alaupovic and colleagues (2, 3, 5, 6). HDL particles containing both apoA-I and apoA-II appear to be the most prevalent lipoprotein species within HDL.

Total HDL mass in normal human plasma is approximately 300 mg/dl (14). HDL has classically been divided into two density classes: HDL_2 (1.063–1.125 g/ml) and HDL_3 (d, 1.125–1.21 g/ml) (15). Recently, three HDL subfractions were isolated within HDL (16). These subfractions had particle size ranges of 8.5–9.6, 9.7–10.7, and 10.8–12.8 nm, with two of these subfractions (HDL_{2b} and HDL_{2a}) being found in HDL_2, while the third subfraction corresponded to HDL_3. A negative correlation between HDL_2 and very low density lipoproteins (VLDL) has been reported (14). Population studies suggest that fluctuations in HDL levels are largely due to changes in HDL_2 (17). Decreased HDL cholesterol levels have been associated with an increased incidence of coronary heart disease in man (18–20).

Familial HDL deficiency (Tangier disease) is a rare inherited disorder originally described in two children from Tangier Island, which is located in the southern Chesapeake Bay (21). These patients presented with enlarged orange tonsils and lymphadenopathy. To our knowledge, since this original report, a total of 27 subjects from 22 kindreds have been reported whose clinical and laboratory findings were consistent with homozygous Tangier disease (22). Clinical features in homozygotes include enlarged orange tonsils, splenomegaly, hepatomegaly, corneal infiltration, lymphadenopathy, and peripheral neuropathy (21–23). Cholesteryl ester deposition in histiocytes has been noted in the tonsils, liver, spleen, lymph nodes, jejunum, rectal mucosa, conjunctiva, and skin of these patients (24–26). Tangier homozygotes have a striking deficiency of HDL, as well as apoA-I and apoA-II, in their plasma (21–23, 27). We have previously reported that these decreased levels are largely due to rapid and altered fractional catabolism of apoA-I and apoA-II (27). In order to test whether this rapid catabolism would persist despite major alterations in the plasma pool size of these proteins, we investigated the disappearance from plasma of HDL constituents (apoA-I, apoA-II, cholesterol, phospholipid, and triglyceride) as well as HDL subfractions (HDL_{2b}, HDL_{2a}, and HDL_3) following the intravenous infusion of large amounts of plasma or HDL into patients with homozygous Tangier disease.

METHODS

Subjects studied

Two patients who were homozygous for familial HDL deficiency were studied. Patient A was a 61-year-old male of weight 82.2 kg and height 173 cm, who was originally described by Hoffman and Fredrickson (28). His fasting plasma cholesterol, triglyceride, and lipoprotein cholesterol concentrations were: cholesterol 74 mg/dl, triglyceride 207 mg/dl, very low density lipoprotein (VLDL) cholesterol 25 mg/dl, low density lipoprotein (LDL) cholesterol 47 mg/dl, and HDL cholesterol 2 mg/dl, as measured by established Lipid Research Clinic procedures utilizing the Autoanalyzer II (Technicon, Tarrytown, NY) (29). Mean normal values ± SEM for our laboratory (n = 1088) are: cholesterol 189 ± 1.2 mg/dl, triglyceride 87 ± 1.3 mg/dl, VLDL cholesterol 16 ± 0.3 mg/dl, LDL cholesterol 123 ± 1.1 mg/dl, and HDL cholesterol 50 ± 0.4 mg% (30). This patient was studied following coronary artery bypass surgery for triple vessel coronary artery disease. During this operative procedure, the patient was on cardiopulmonary bypass and received nine units of blood, resulting in an exchange of his plasma with normal plasma. Blood was collected in 0.1% EDTA from this patient prior to surgery, and at 4, 8, 12, 16, 40, and 64 hr following the completion of the procedure (approximate duration 5 hr).

Patient B was a 23-year-old white female and a member of the original kindred from Tangier Island as described by Fredrickson et al. (21). Her weight and height were 72.1 kg and 180 cm, respectively. Her fasting plasma lipid and lipoprotein cholesterol values were as follows: cholesterol 51 mg/dl, triglyceride 274 mg/dl, VLDL cholesterol 20 mg/dl, LDL cholesterol 29 mg/dl, and HDL cholesterol 2 mg/dl. This patient was placed on an isocaloric diet containing 20% protein, 40% fat, 40% carbohydrate, 300 mg cholesterol/day, with a normal polyunsaturated to saturated fat ratio (0.2) before and during each of two metabolic studies. Weight fluctuated by less than ± 0.5 kg during each study. At the beginning of the first study, the patient received ^{125}I-labeled apoA-I (98 μCi) and ^{131}I-labeled apoA-II (24 μCi) intravenously. Following injection, blood was drawn at 10 min, 30 min, 1, 3, 6, 9, 12, 24, 48, and 72 hr in 0.1% EDTA. In addition, this patient was studied in an identical manner following an infusion of HDL to raise the plasma con-

centration of HDL to near normal levels. The time between studies was 9 months. HDL for infusion was isolated from the plasma of five normal volunteers with normal lipoprotein levels, no history of hepatitis, transfusions, or recent illness, a negative serum hepatitis associated antigen test, and a negative serum fluorescent treponemal antibody test. Each volunteer underwent a two-unit plasmapheresis in 0.1% EDTA, yielding 6.15 liters of plasma from 10 liters of blood which was utilized for the preparation of HDL. The HDL was administered to patient B, intravenously, at the beginning of the second study, in a volume of 425 ml (protein concentration, 9.36 mg/ml) over an 18-min period. Radiolabeled [^{125}I]-apoA-I (96 μCi) and [^{131}I]apoA-II (22 μCi) were given intravenously immediately following the end of the HDL infusion, and blood samples were drawn in 0.1% EDTA at 5 min, 30 min, 1, 3, 6, 9, 12, 24, 48, and 72 hr after injection of the tracers.

A 21-year-old normal female control subject (height, 158 cm; weight 67.8 kg) was also studied and received the same radiolabeled apolipoprotein preparations (55 μCi ^{125}I-labeled apoA-I; 26 μCi ^{131}I-labeled apoA-II) intravenously, as did patient B during her baseline study. This subject was on the same isocaloric balanced diet as was patient B. Weight fluctuated by less than ± 0.5 kg during the study. Plasma lipid and lipoprotein cholesterol levels were: cholesterol 141 mg/dl, triglyceride 85 mg/dl, VLDL cholesterol 16 mg/dl, LDL cholesterol 72 mg/dl, and HDL cholesterol 53 mg/dl. Blood was collected in 0.1% EDTA at 10 min and 6 and 12 hr after injection of the tracers and then daily for 14 days. All subjects undergoing tracer studies were given supersaturated potassium iodide (SSKI) 1 g/day and ferrous gluconate 900 mg/day, 3 days prior to and throughout the course of each study. All subjects had normal thyroid, renal, and kidney function. Informed consent was obtained from all subjects.

Isolation and radioiodination of apolipoproteins

ApoA-I and apoA-II were isolated from normal HDL (density 1.09–1.21 g/ml) by column chromatography as previously described (8, 11). These apolipoproteins formed discrete bands on tetramethylurea and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (31, 32). Each apolipoprotein formed a single immunoprecipitin line with appropriate antisera when tested with antisera for apoA-I, apoA-II, apoB, apoC-II, apoC-III, apoE, and albumin. Apolipoproteins A-I and A-II were iodinated in sterile 1 M glycine, 0.1 M Tris, pH 8.5 buffer by the iodine monochloride method of McFarlane (33). Free iodine was removed by extensive dialysis against sterile 0.85% NaCl, 0.01% EDTA, 0.1 M Tris, pH 7.4 buffer. Less than 1% free iodine was detectable by 20% trichloroacetic acid precipitation, and none was noted on paper radiochromatography in 85% methanol using No. 1 Whatman filter paper. In order to minimize radiation damage, all radiolabeled apoprotein preparations were diluted 1:10 with 5% sterile human albumin solution prior to use in metabolic studies. The mean efficiency of iodination (±SD) was 68 ± 11% for apoA-I, and 51 ± 7% for apoA-II. A mean of 0.6 ± 0.2 moles of iodine was incorporated per mole of protein with each iodination. When radiolabeled and unlabeled apoA-I and apoA-II were chromatographed (columns 1.2 × 150 cm glass; buffer, 0.05 M Tris, 6 M urea, pH 8.2), on Sephadex G-200 (superfine), radioactivity and protein as measured by absorbance (280 nm) eluted as single superimposable peaks. A mean of 91.5 ± 4.1% of apoA-I radioactivity and 95.2 + 1.2% apoA-II radioactivity was recovered in the appropriate gel band on tetramethylurea polyacrylamide gel electrophoresis. Prior to injection all radiolabeled apolipoproteins were subjected to Millipore filtration and were found to be sterile and pyrogen-free.

Isolation characterization, and quantitation of apolipoprotein and lipoprotein constituents

Blood samples obtained during metabolic studies were centrifuged at 4°C to obtain plasma. High density lipoprotein cholesterol was measured in plasma samples by heparin manganese precipitation (29). Apolipoprotein A-I and A-II levels were measured in whole plasma and HDL by electroimmunoassay as previously described (34), except that samples were delipidated with methanol and diethylether prior to assay (35). Mean normal plasma values (±SD) were 131.0 ± 16.0 mg/dl for apoA-I and 28.0 ± 2.8 mg/dl for apoA-II. Very low density lipoproteins (d < 1.006 g/ml), LDL (1.006–1.063 g/ml), and HDL (1.063–1.21 g/ml) were isolated by sequential ultracentrifugation, utilizing a Beckman 40.3 rotor and a Beckman L265B ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA) at 39,000 rpm (36).

High density lipoproteins (1.063–1.21 g/ml) for the infusion study were isolated from 6.15 liters of plasma using sterile technique in Beckman 60 Ti rotors by sequential ultracentrifugation at 59,000 rpm (36). Potassium bromide (KBr) was used for density adjustments. The isolated HDL was extensively dialyzed against 0.85% Nacl, 0.01% EDTA to remove the KBr. The preparation was then Millipore-filtered,

TABLE 1. Apolipoprotein A-I, A-II, and high density lipoprotein cholesterol[a]

	Plasma		High Density Lipoproteins[b]		
Time	ApoA-I	ApoA-II	ApoA-I	ApoA-II	Cholesterol
hours			*mg/dl*		
Patient A					
Pre	2.1 ± 0.7	2.5 ± 0.9	0.8 ± 0.2	0.3 ± 0.1	2
4	68.8 ± 1.2	18.0 ± 1.3	61.4 ± 2.1	15.2 ± 1.0	20
8	55.8 ± 2.1	16.3 ± 1.1	49.4 ± 2.4	16.1 ± 1.1	16
12	47.5 ± 2.5	15.8 ± 1.0	43.2 ± 1.7	9.4 ± 0.9	15
16	34.8 ± 1.6	8.8 ± 1.4	24.5 ± 1.2	6.7 ± 0.8	13
40	8.2 ± 1.0	4.5 ± 0.8	7.6 ± 0.9	3.3 ± 0.9	4
64	3.1 ± 0.8	2.4 ± 0.6	0.9 ± 0.4	0.2 ± 0.2	2
Patient B					
Baseline study	1.8 ± 1.0	2.8 ± 1.0	0.8 ± 0.1	0.4 ± 0.1	2
Pre-infusion	1.8 ± 1.0	2.8 ± 1.0	0.7 ± 0.1	0.3 ± 0.1	1
Normal Subject	125.1 ± 4.5	23.0 ± 1.5	118.2 ± 5.2	22.0 ± 2.1	53

[a] Values given as mean ± SD.
[b] Heparin manganese supernatant.

and sealed under sterile conditions, and was noted to be sterile and pyrogen-free. Cholesterol and triglyceride in plasma and lipoprotein fractions were measured by Autoanalyzer II methodology (29), phospholipids by the method of Chalvardjian and Rudnicki (37), and protein by the method of Lowry et al. (38).

Plasma from all time points during the infusion study was subjected to paper lipoprotein electrophoresis (29). In addition, plasma samples were shipped on ice to the Donner Laboratory, Berkeley CA for analytical ultracentrifugation (39). High density lipoprotein subfractions HDL_{2b}, HDL_{2a}, and HDL_3 were quantitated utilizing the method of Anderson et al. (17). In all metabolic studies, radioactivity was quantitated with a Packard Model 3375 gamma counter (Packard Instrument Co., Downers Grove, IL).

Kinetic analysis

Radioactivity and specific radioactivity decay curves, as well as the disappearance rate of plasma and HDL apoA-I and A-II, HDL subfractions and HDL cholesterol, triglyceride, phospholipids, and protein were assessed by standard least squares curve-fitting techniques using single or multiple exponential analysis utilizing the SAAM computer program (40). The residence times were calculated from the area under the decay curve (41, 42). All curves could be fit with either one, two, or three exponentials. For radiolabeled apolipoprotein studies in the baseline state, the synthesis rate in mg/day was calculated by dividing the plasma mass of the given apolipoprotein by the residence time (42). Assumptions made are that the patient's apolipoprotein levels were in a steady state, and that all apolipoprotein synthesis enters the plasma space (42). With regard to the decay rates of HDL constituents following HDL infusion into patient B, decay curves were fit to one or more exponentials, with the zero value being given to the preinfusion value for a given constituent. The best fit (least squares) for HDL triglyceride, phospholipid and protein was obtained using a single exponential, while for HDL cholesterol, two exponentials were required.

RESULTS

The disappearance of plasma and HDL apoA-I and apoA-II as well as HDL cholesterol in patient A is shown in **Table 1.** Since plasma was not obtained immediately after the cessation of cardiopulmonary bypass, and because the patient received blood after coming off bypass, no accurate peak values for HDL constituents were obtained. Therefore no kinetic analysis of the disappearance of HDL constituents was undertaken. However, as can be seen, there was rapid disappearance of HDL so that by 64 hr after the surgical procedure the concentration of apoA-I, apoA-II, and HDL cholesterol were very similar to preoperative levels. In addition, while most of the apoA-I and apoA-II was in the heparin-manganese supernate following plasma exchange, prior to plasma exchange and at the 64-hr point, over 50% of both plasma apoA-I and apoA-II were removed by heparin-manganese precipitation. Similar observations were noted in patient B in the baseline state where 58% of plasma apoA-I and 84% of plasma apoA-II were removed by heparin-manganese precipitation (see Table 1). In contrast, over 90% of both plasma apoA-I

and apoA-II were found in the HDL fraction (heparin-manganese supernate) in normal plasma or after plasma exchange (see Table 1).

In the normal subject and patient B during baseline studies, the mean plasma apoA-I and apoA-II levels are given in Table 1. The percentages of plasma apoA-I and apoA-II radioactivity associated with lipoprotein density fractions are shown in **Table 2**. In the normal subject, almost all the radioactivity was associated with HDL. In patient B, a significant amount of apoA-I radioactivity was in VLDL and LDL as well in the 1.21 g/ml infranate, while most of the apoA-II radioactivity was found in VLDL and LDL.

The decay of radioiodinated apoA-I and apoA-II following intravenous injection in the normal subject and patient B (in the baseline study) are shown in **Fig. 1.** As can be seen, radiolabeled apoA-I and apoA-II decay was significantly more rapid in the homozygote than in the normal control. In both the normal subject and the homozygote, apoA-I and apoA-II decay curves were best fit with three exponentials. The residence times derived from the areas under the decay curves were 4.04 days for apoA-I and 4.44 days for apoA-II in the normal subject, and 0.22 days for apoA-I and 0.81 days for apoA-II in the homozygote. Synthesis rates in mg/kg per day for apoA-I and apoA-II were 11.82 and 1.99 in the normal subject, and 3.81 and 1.61 in the homozygote, respectively.

HDL was infused into patient B, and radiolabeled [^{125}I]apoA-I and [^{131}I]apoA-II were injected at the end of infusion. Following the infusion there was a rapid disapperance of apoA-I and apoA-II from both plasma and HDL as can be seen in Table 5; so that by day 3 the concentrations of these apolipoproteins were similar to preinfusion levels. The residence times for plasma and HDL apoA-I were 0.63 days and 0.31 days, respectively, and for plasma and HDL apoA-II were 1.88 days and 0.83 days, respectively. The apo A-I and apoA-II radioactivity decay in plasma was significantly faster than in the normal control, and the terminal slopes of the preinfusion and postinfusion curves were quite similar. However, increasing the HDL mass in patient B did appear to retard the initial decay of both tracers as compared to the baseline study (Fig. 1).

Specific activity decay curves for apoA-I and apoA-II decay following HDL infusion are shown in **Fig. 2.** In both plasma and HDL there was an initial drop for apoA-I with a subsequent slight rise and then another slight drop. For apoA-II, an initial period of little change in specific activity was seen with a subsequent slow decline. The distribution of apoA-I and apoA-II radioactivity is given in Table 2. Initially, after infusion, almost all apoA-I and apoA-II radioactivity was found within the HDL density range. However, following the decay of HDL constituents, increasing amounts of radioactivity were found in the 1.063 g/ml supernate (especially apoA-II) and in the 1.21 g/ml infranate (especially apoA-I), reflecting the mass distribution of these apolipoproteins among plasma lipoprotein density fractions.

Following the infusion of HDL in patient B, alpha and beta lipoprotein bands could be seen on lipoprotein electrophoresis. Gradually both bands dis-

TABLE 2. Lipoprotein density distribution of [^{125}I]-ApoA-I and [^{131}I]-ApoA-II radioactivity[a]

	[^{125}I]-ApoA-I				[^{131}I]-ApoA-II			
	VLDL	LDL	HDL	1.21B[b]	VLDL	LDL	HDL	1.21B[b]
	% of total							
Normal Subject	0.6	0.2	93.1	6.1	0.3	0.2	95.0	4.5
Patient B								
Baseline study	15.7	15.5	21.2	47.6	39.2	42.4	11.6	6.8
Infusion study								
Incubation[c]	17.3	14.7	22.5	45.5	39.9	34.4	14.3	11.4
5 min	0.8	0.7	90.3	8.2	0.9	0.8	94.9	3.4
30 min	0.7	0.5	90.1	8.7	0.6	0.7	95.9	2.8
3 hr	1.8	1.3	87.2	9.7	0.8	0.8	96.6	1.8
6 hr	1.0	0.7	87.4	10.9	0.5	0.6	94.6	4.3
9 hr	3.0	2.8	86.5	7.7	1.0	1.2	95.7	2.1
12 hr	3.6	3.1	85.8	7.5	1.2	1.1	95.5	2.2
24 hr	6.4	4.3	79.3	10.0	22.2	1.4	73.2	3.2
48 hr	9.6	8.4	46.5	35.5	19.9	5.0	63.0	12.1
72 hr	18.7	20.2	21.4	39.7	39.8	22.4	27.1	10.7

[a] Fractional standard deviations on all determinations were less than 0.05.
[b] 1.21B is the 1.21 g/ml infranate.
[c] Distribution of radioactivity among lipoprotein fractions in plasma of patient B obtained prior to infusion and incubated at 37°C for 30 min in a shaking water bath with trace amounts of [^{125}I]-apoA-I and [^{131}I]-apoA-II.

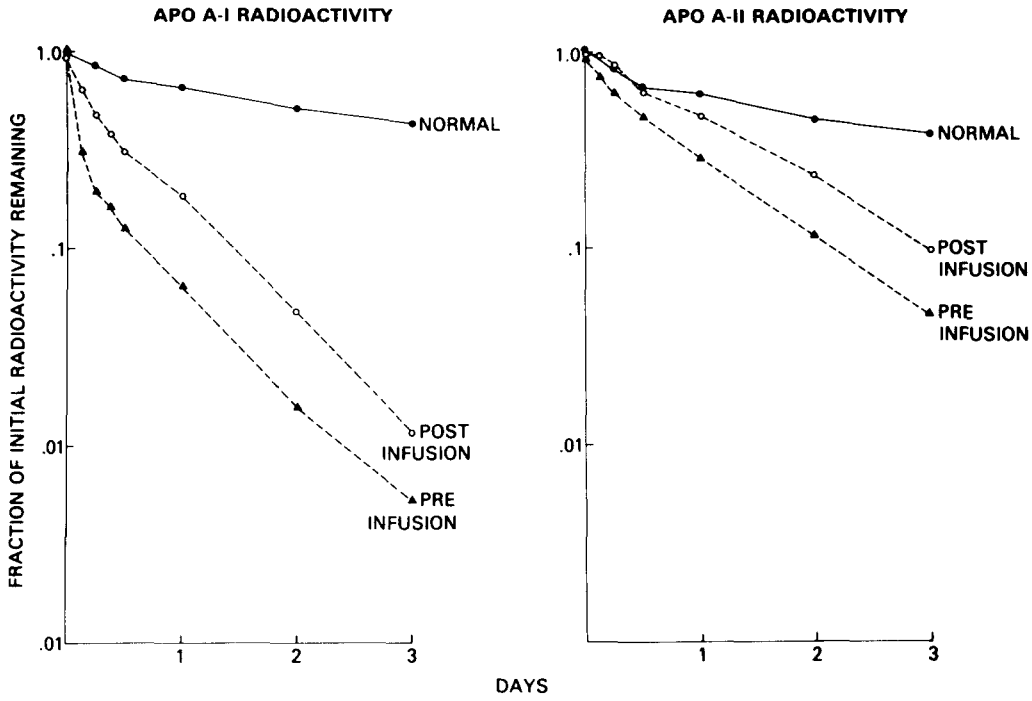

Fig. 1. Plasma radioactivity decay curves for ^{125}I-apoA-I and ^{131}I-apoA-II in a normal subject and in patient B before and after HDL infusion. Radiolabeled apolipoproteins were injected in lipoprotein-free form so they could associate with all apoA-I and apoA-II containing lipoproteins in plasma. The amount of initial radioactivity is based on the plasma sample obtained within 10 min after injection. All points are plotted except for the 30-min point which was almost identical to the initial time point.

appeared, and only a prebeta-lipoprotein band was seen, identical to the lipoprotein electrophoretic pattern seen in patient B in the baseline state. The alpha lipoprotein band was last distinctly seen at 24 hr after infusion and at that time its electrophoretic mobility was slightly faster than at the 5-min point. Plasma lipid levels and VLDL and LDL compositional data are given in **Table 3** for patient B, prior to and following the HDL infusion. An immediate rise in plasma cholesterol was noted following the infusion due to an increase in HDL cholesterol. Plasma triglyceride levels did not change significantly after infusion, except for an isolated rise at 3 days which may have been due to dietary indiscretion. There was a drop in LDL triglyceride content with a rise in HDL triglyceride levels as can be seen in Table 3. Slight decreases in LDL protein and phospholipid were also noted following HDL infusion, with subsequent reversion of these LDL constituents as well as triglycerides to preinfusion levels by day 3. These alterations may have been due to the transfer of some LDL constituents to HDL following HDL infusion, and may account for the alterations in LDL electrophoretic mobility seen following HDL infusion.

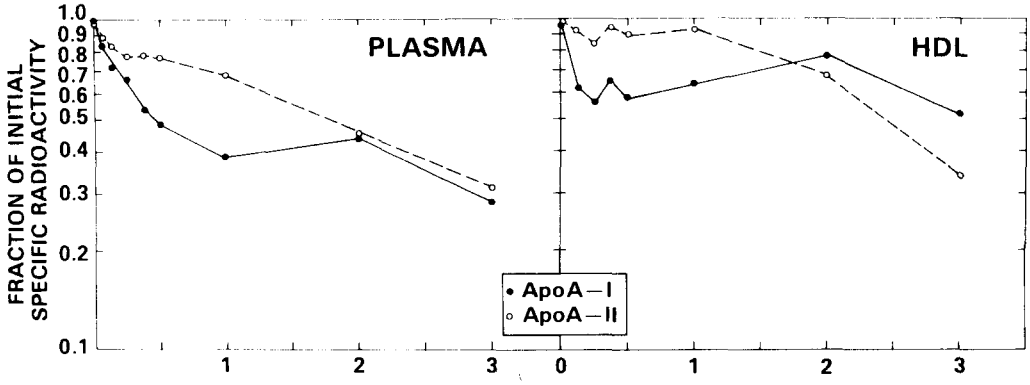

Fig. 2. ^{125}I-apoA-I and ^{131}I-apoA-II specific activity decay in patient B after HDL infusion. All points are plotted except for the 30-min point which was almost identical to the initial time point.

TABLE 3. Plasma lipid and lipoprotein analyses[a]: Patient B

Time	Plasma		Very Low Density Lipoproteins				Low Density Lipoproteins			
	Cholesterol	Triglyceride	Cholesterol	Triglyceride	Phospholipid	Protein	Cholesterol	Triglyceride	Phospholipid	Protein
					mg/dl					
Pre	53 ± 2	274 ± 11	18 ± 1	210 ± 6	57 ± 1	22 ± 0	27 ± 1	61 ± 2	44 ± 1	47 ± 1
5 min	91 ± 3	273 ± 12	18 ± 1	207 ± 12	43 ± 4	18 ± 1	29 ± 0	41 ± 1	45 ± 2	43 ± 0
30 min	94 ± 1	261 ± 10	19 ± 1	197 ± 9	36 ± 4	21 ± 1	34 ± 2	42 ± 0	47 ± 1	42 ± 1
3 hr	91 ± 3	271 ± 7	19 ± 1	211 ± 3	45 ± 4	23 ± 1	32 ± 2	31 ± 2	33 ± 2	38 ± 1
6 hr	86 ± 2	281 ± 11	19 ± 2	232 ± 2	52 ± 3	25 ± 1	31 ± 1	24 ± 1	34 ± 3	38 ± 1
9 hr	84 ± 1	276 ± 13	20 ± 1	231 ± 4	48 ± 4	24 ± 1	31 ± 4	21 ± 1	37 ± 3	36 ± 1
12 hr	79 ± 2	263 ± 8	18 ± 2	224 ± 8	49 ± 2	21 ± 1	29 ± 2	22 ± 2	38 ± 1	35 ± 0
24 hr	76 ± 1	228 ± 6	17 ± 1	184 ± 4	56 ± 3	21 ± 1	32 ± 3	28 ± 2	35 ± 3	35 ± 1
48 hr	75 ± 2	280 ± 9	21 ± 1	219 ± 8	65 ± 1	26 ± 1	35 ± 2	53 ± 3	51 ± 1	47 ± 0
72 hr	69 ± 2	364 ± 12	26 ± 1	290 ± 8	78 ± 3	35 ± 1	29 ± 2	64 ± 3	56 ± 1	52 ± 1

[a] Mean values ± SD.

Quantitation of plasma lipoproteins by analytical ultracentrifugation are shown in **Table 4.** A drop in low density lipoproteins levels (S_f 0–12, 12–20) were seen during the infusion, with a striking rise in HDL. High density lipoprotein levels were slightly lower at 5 min after infusion than they were at the 30-min point, probably due to an increased plasma volume at the 5-min point secondary to the infusion itself. The infused HDL had a somewhat higher percentage of HDL_{2b} and HDL_{2a} than is normally seen, possibly because the HDL was isolated from young lean males and females with slightly increased HDL cholesterol values (mean 61 mg/dl). The HDL_{2b} subfraction was no longer detectable at the 9-hr point, while HDL_{2a} and HDL_3 could still be detected at the 24-hr point but not thereafter. Peak levels for HDL_{2b} were seen at 30 min, for HDL_{2a} at 1 hr, and for HDL_3 at 6 hr. The residence times for HDL_{2b}, HDL_{2a}, and HDL_3 decay were 0.1, 0.8, and 2.7 days, respectively. In each case the mass decay was fit to a single exponential from the peak value to the 24-hr point. Such analysis assumes that there is no further input into an HDL subfraction after the peak value. This assumption may not be a valid one, especially for HDL_3, if there is significant conversion of HDL_{2b} and HDL_{2a} to HDL_3. By 48 hr no HDL could be detected by analytical ultracentrifugation and by compositional analysis HDL constituents (**Table 5**) were close to preinfusion values. These data would suggest a more rapid disappearance of HDL_3 between 24 and 48 hr, than in the initial 24 hr following HDL infusion. This observation could be explained by either an increase in the residence time of HDL_3 after 24 hr as the pool size decreased, or due to decreased input into HDL_3 from HDL_{2b} and HDL_{2a} during the latter time period. We favor the latter alternative because the decay of HDl protein, phospholipid, and tryglyceride was monoexponential throughout this experiment.

Plasma apoA-I and apoA-II levels, as well as HDL apoA-I, A-II, cholesterol, triglyceride, phospho-

TABLE 4. Analytical ultracentrifugation lipoprotein patterns: Patient B

	Low Density Lipoproteins S_f^0				High Density Lipoproteins			
	100–400	20–100	12–20	0–12	HDL_{2b}	HDL_{2a}	HDL_3	Total
				mg/dl				
HDL prep[a]	0	0	0	0	31	119	77	227
Pre	64	85	13	184	0	0	0	0
5 min	26	47	0	111	10	113	72	195
30 min	58	98	18	159	38	136	89	263
1 hr	91	95	13	170	32	148	90	270
3 hr	10	95	0	126	7	98	89	194
6 hr	26	118	15	140	6	125	106	237
9 hr	46	99	23	122	0	95	94	189
12 hr	11	74	11	117	0	61	94	155
24 hr	53	82	20	119	0	48	85	133
48 hr	76	121	48	178	0	0	0	0
72 hr	84	137	33	158	0	0	0	0

[a] Infused HDL, diluted 1:7 with normal saline.

TABLE 5. Apolipoprotein A-I and A-II concentration and high density lipoprotein composition after HDL infusion:[a] Patient B

Time	Plasma		High Density Lipoproteins						
	ApoA-I	ApoA-II	ApoA-I	ApoA-II	Protein	Alpha Cholesterol	Cholesterol	Phospho-lipid	Tri-glyceride
					mg/dl				
Pre	1.8 ± 1.0	2.8 ± 1.1	trace	trace	18 ± 1	1	2 ± 1	12 ± 1	0 ± 0
5 min	98.4 ± 6.2	32.1 ± 1.2	95.1 ± 6.3	35.2 ± 3.0	113 ± 8	28	24 ± 1	63 ± 1	15 ± 2
30 min	98.7 ± 6.1	35.2 ± 2.3	95.6 ± 8.0	31.2 ± 3.1	117 ± 10	27	27 ± 1	65 ± 1	19 ± 1
1 hr	101.7 ± 4.4	37.1 ± 2.1	ND	ND	ND	ND	ND	ND	ND
3 hr	88.2 ± 2.0	35.2 ± 3.6	94.7 ± 7.6	27.8 ± 3.2	109 ± 12	23	28 ± 1	67 ± 1	23 ± 2
6 hr	73.1 ± 2.1	30.9 ± 8.2	80.4 ± 3.7	26.2 ± 3.7	92 ± 4	17	19 ± 1	60 ± 2	20 ± 1
9 hr	69.3 ± 1.8	28.2 ± 3.7	51.9 ± 2.0	13.6 ± 2.2	87 ± 3	16	17 ± 2	52 ± 1	16 ± 2
12 hr	61.8 ± 4.1	24.8 ± 6.4	47.7 ± 3.1	11.2 ± 2.4	78 ± 7	14	13 ± 1	47 ± 1	11 ± 2
24 hr	46.6 ± 6.3	22.1 ± 1.4	24.2 ± 1.2	10.1 ± 2.7	65 ± 2	12	13 ± 2	40 ± 1	10 ± 2
48 hr	10.7 ± 4.2	16.6 ± 2.3	1.1 ± 1.1	8.1 ± 1.4	24 ± 1	2	3 ± 1	15 ± 1	1 ± 1
72 hr	3.8 ± 1.9	9.6 ± 1.1	0.5 ± 0.5	7.7 ± 2.1	20 ± 4	0	2 ± 1	13 ± 1	0 ± 0

[a] Mean values are given with standard deviations. Standard deviations could not be determined for alpha cholesterol since only one determination at each time point was carried out. Alpha cholesterol is HDL cholesterol determined by heparin-manganese precipitation of plasma. ND, not determined.

lipid, and protein concentrations, prior to and following HDL infusion in the patient B are shown in Table 5. By day 3 following the infusion, almost all HDL constituents were back to baseline values. High density lipoprotein and phospholipid both had a monoexponential decay with a half-life of 0.7 days, while HDL triglyceride disappeared monoexponentially with a slightly faster half-life of 0.5 days. High density lipoprotein cholesterol had a biexponential decay, with an initial fast component and a subsequent slow component with a residence time of 0.7 days.

DISCUSSION

Patients with homozygous familial HDL deficiency (Tangier disease) have very low levels of apoA-I, apoA-II, and other HDL constituents in their plasma (13, 21–23, 27, 43, 44). The mean apoA-I and A-II plasma concentrations of the two homozygotes in this investigation were 2% and 10% of normal, respectively. We have previously reported that 28% of plasma apoA-I and 92% of plasma apoA-II mass was associated with the 1.063 g/ml supernate in these patients (as determined after ultracentrifugation in a Beckman 40.3 rotor at 39,000 rpm) (27). In patient B in the baseline study, a mean of 31.2% of apoA-I radioactivity and 81.6% of apoA-II radioactivity was noted in the 1.063 g/ml supernate, consistent with the concept that the distribution of radiolabeled apoA-I and apoA-II among lipoproteins was similar to that of native apolipoproteins.

It has been reported that apoA-I is readily dissociated from lipoproteins by ultracentrifugation (45). In addition, the self-association of apoA-I in aqueous solution is greatly affected by changes in concentration as well as in pressure (9, 46). We therefore examined apoA-I and apoA-II mass distribution among lipoproteins by heparin-manganese precipitation. In these experiments we found that a mean of 60% of apoA-I and 86% of apoA-II were removed from Tangier plasma by heparin-manganese precipitation. In contrast, over 90% of both plasma apoA-I and apoA-II were found in the HDL fraction (heparin-manganese supernate) in normal plasma or in Tangier plasma just after HDL infusion. Assmann et al. (43) and Henderson et al. (44) have reported that over 95% of the apoA-I in Tangier plasma is found in the 1.21 g/ml infranatant after ultracentrifugation (Beckman 60 Ti, rotor at 59,000 rpm). The differences in these data and our own appear to relate to variability in isolation techniques. Our experiments indicate that a significant fraction of plasma apoA-I and apoA-II is associated with apoB containng lipoproteins (i.e., VLDL and LDL) in Tangier homozygotes.

Analysis of baseline metabolic studies in patient B suggested that apoA-I and apoA-II residence times obtained with radiolabeled apoA-I and apoA-II were very similar to those obtained with radiolabeled HDL as previously reported (27). Patient B had apoA-I and apoA-II synthesis rates (mg/kg per day) that were 32% and 83%, respectively, of those obtained in the normal subject using the same tracer preparations. ApoA-I and apoA-II residence times in patient B were 5.4% and 18.3% of normal, respectively. These data indicate that this Tangier homozygote had both markedly enhanced fractional catabolic rates

of plasma apoA-I and apoA-II, as well as a moderate decrease in the synthetic rates of those two apolipoproteins.

In order to examine whether this rapid catabolism of apoA-I and apoA-II could be a function of a decreased plasma pool size, we increased the pool size of these proteins as well as other HDL constituents. Patient A underwent a complete plasma exchange on cardiopulmonary bypass during coronary artery surgery. In this patient, apoA-I, apoA-II, and HDL cholesterol plasma values within 64 hr after the completion of surgery were similar to those obtained prior to surgery. Similar results were noted in another Tangier patient who had undergone a pulmonary valve replacement with cardiopulmonary bypass at the National Institutes of Health (47). In patient B, there was a rapid disappearance of HDL constituents as well as apoA-I and apoA-II radioactivity from plasma following an HDL infusion, so that by 72 hr plasma apoA-I and apoA-II concentrations were similar to pre-infusion values. These data are consistent with the concept that the rapid catabolism of apoA-I and apoA-II in Tangier disease persists, despite major increases in the plasma pool size of these proteins. It should be noted that the decay of apoA-I and apoA-II radioactivity was somewhat slower for the first 6 hr after infusion than in the baseline study during the same time period. Thereafter, the decay was similar for the two tracers in both studies. Therefore, an increase in pool size retarded the initial decay but not the terminal decay of these two tracers. To test more adequately the effect of pool size changes on the catabolism of HDL apolipoproteins, one would have to do a clamp experiment maintaining the HDL pool-size at a normal level for a given period of time by continuous infusion. At the present time this is not feasible.

Following the infusion of HDL and radiolabeled apoA-I and apoA-II into patient B, we noted a drop, then a slight rise, and then a subsequent further decrease in apoA-I specific radioactivity. This initial fall and subsequent rise were probably due to radioactivity leaving the plasma space with subsequent return at a later time. The amount of infused HDL in patient B was sufficient to raise her HDL plasma mass to at least one and one-half normal levels (based on a plasma volume of 3010 ml in the baseline study determined by isotope dilution). Since normal levels of HDL were not quite achieved in patient B, a significant fraction of the infused HDL must have left the plasma space almost immediately after infusion. These data are compatible with the metabolic model for HDL proposed by Blum et al. (48) which consisted of a plasma and a non-plasma pool, with exchange between these pools. In addition, Anderson et al. (49) have proposed that apoA-I and apoA-II on lipoproteins can recirculate between plasma and lymph based on 24-hr thoracic duct lymph output studies in man.

Recently, Assmann et al. (50) reported a rapid disappearance of plasma apoA-I, apoA-II, and HDL cholesterol following an intravenous infusion of HDL and ^{125}I-labeled HDL into one Tangier homozygote. These investigators noted a steady decay of apoA-II specific radioactivity within the 1.006–1.21 g/ml density region; however, no change in apoA-I specific radioactivity within this same density region was detected throughout the course of the experiment. These data were interpreted to indicate that newly synthesized apoA-I was not entering this density region and therefore no drop in apoA-I specific radioactivity was noted. An alternative explanation would be that the apoA-I concentrations in this density region in the baseline state in Tangier plasma were so low following isolation of lipoproteins that no dilution of specific radioactivity was detectable. Since Tangier plasma does contain detectable amounts of apoA-I and apo-II in the LDL and HDL density region in the baseline state, newly synthesized apoA-I and apoA-II must ultimately enter these lipoprotein density regions irrespective of the form in which these proteins enter plasma (27). The reason for the differences in our data and that obtained by Assmann et al. (50) are not clear.

An HDL infusion in a Tangier homozygote provides one with a unique opportunity to study the catabolism of HDL constituents and subfractions. Alterations in HDL concentrations in man are largely due to fluctuations in HDL_2 levels (17). Subjects with high levels of HDL have a significant amount of HDL_{2b}. Subjects with intermediate HDL levels have mainly HDL_{2a} and HDL_3, while individuals with low HDL concentrations have almost exclusively HDL_3 particles (17). Attempts to study the metabolism of HDL subfractions in man, utilizing radiolabeled apolipoproteins or lipoproteins, have been hampered by rapid exchange of radiolabeled apolipoproteins among subfractions (4, 51). In the present experiment, the more lipid-rich HDL subfractions were catabolized at a greater fractional rate than was HDL_3. In addition, there may have been some interconversion of HDL_{2b} and HDL_{2a} to HDL_3, since between 1 hr and 6 hr after infusion, HDL_{2b} and HDL_{2a} decreased 82% and 16%, respectively, while HDL_3 increased by 18%. These changes appeared to occur because HDL cholesterol and triglyceride were removed at a faster rate than protein and phospholipid,

suggesting that HDL core constituents may be catabolized at a slightly faster rate than surface components. In addition, apoA-I was catabolized at a faster rate than apoA-II in the infusion experiment, and this appears to be the case in normal man as well (52). If the catabolism of HDL particles proceeds in normal man in the same manner as in the infusion study, then an explanation for the varying particle size, lipid content, and apoA-I:apoA-II ratio among HDL subfractions would be provided. It has been reported that the highest ratio of apoA-I:apoA-II is found in the most lipid-rich HDL subfractions, and the lowest in very small HDL particles (1, 4, 53). In this view, subjects with the lowest HDL levels would be predicted to have very lipid-poor, small HDL particles in the HDL_3 density range with a very low apoA-I:apoA-II ratio. Indeed such particles have been isolated in homozygous familial HDL deficiency (47).

Tangier homozygotes clearly synthesize significant amounts of apoA-I and apoA-II (27). The intestine appears to serve as a major source of these apolipoproteins in man. The presence of both apoA-I and apoA-II in both normal and Tangier intestinal mucosa has been documented with immunological techniques (54, 55).With fat feeding, an increase in apoA-I immunofluorescence in Tangier intestine epithelial cells has been noted, as has a subsequent rise in plasma apoA-I (55). Human lymph chylomicron apoA-I and apoA-II can serve as precursors for these constituents within plasma HDL, and this conversion appears to be defective in Tangier homozygotes, resulting in rapid and altered catabolism of apoA-I and apoA-II within VLDL and LDL (27, 56, 57). The cholesteryl ester deposits noted in the reticuloendothelial cells of Tangier patients may well be the result of abnormal uptake of intestinal lipoprotein remnants (24). Whether the defect in Tangier disease is due to an abnormality of enzyme activity, receptor function, or a structural abnormality of apoA-I or apoA-II, remains to be elucidated.

The data presented in this manuscript provide further support for the concept that patients with Tangier disease have rapid and altered apoA-I and apoA-II catabolism which persists despite major increases in the HDL plasma pool size. In addition, following the infusion of HDL into a Tangier homozygote, HDL_{2b} and HDL_{2a} disappeared at a faster rate from plasma than HDL_3. These alterations were accompanied by a somewhat faster catabolism of HDL cholesterol and triglyceride than HDL protein and phospholipid, while apoA-I was catabolized at a greater fractional rate than apoA-II. These latter observations may have important implications with regard to the catabolism of HDL subfractions and constituents in normal man.

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