Impaired plasma cholesteryl ester transfer with accumulation of larger high density lipoproteins in some families of baboons (*Papio* sp.)

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Abstract Baboons from some families have a higher concentration of plasma high density lipoproteins (HDL) on a chow diet and accumulate large HDL (HDL_1) when challenged with a high cholesterol and high saturated fat (HCHF) diet. HDL_1 from high HDL_1 animals contained more (1.5-fold) cholesteryl ester than HDL (HDL_2 + HDL_3) from high or low HDL_1 animals. HDL from high HDL_1 baboons had lower triglyceride content than that from low HDL_1 baboons. HDL_3 or HDL labeled with [^{3}H]cholesteryl linoleate was incubated with entire lipoprotein fraction ($d<1.21$ g/ml) or very low density lipoprotein + low density lipoprotein (VLDL + LDL) ($d<1.045$ g/ml) and with lipoprotein-deficient serum (LPDS), and the radioactive cholesteryl ester and mass floating at d 1.045 g/ml (VLDL + LDL) after the incubation was measured. The transfer of cholesteryl esters from either HDL or HDL_3, prepared from plasma of high HDL_1 animals fed chow or the HCHF diet, was slower than the transfer from either HDL or HDL_3 of low HDL_1 animals, regardless of the source of transfer activity or the ratio of LDL:HDL-protein used in the assay. Addition of HDL from high HDL_1 baboons into an assay mixture of plasma components from low HDL_1 baboons decreased the transfer of cholesteryl ester radioactivity and mass from HDL to VLDL and LDL. In addition to HDL, a fraction of intermediate density lipoprotein (IDL) and denser HDL were also effective in inhibiting the transfer. These observations suggest that accumulation of HDL_1 in high HDL_1 baboons fed an HCHF diet is associated with a slower transfer of cholesteryl esters from HDL to LDL. This slower transfer of cholesteryl esters appears to be related to HDL protein composition rather than differences in transfer protein activity.—**Kushwaha, R. S., D. L. Rainwater, M. C. Williams, G. S. Getz, and H. C. McGill, Jr.** Impaired plasma cholesteryl ester transfer with accumulation of larger high density lipoproteins in some families of baboons (*Papio* sp.) *J. Lipid Res.* 1990. **31:** 965–973.

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High density lipoproteins (HDL) have been divided into three major subclasses (HDL_1, HDL_2, and HDL_3) on the basis of their flotation rates (1). These subclasses of HDL are heterogeneous in particle size and lipid and protein composition (2–4). Plasma levels of these lipoprotein subpopulations are affected by age, sex (5, 6), diet and drugs (7–10), exercise (11), and hypertriglyceridemia (12). Among some families of baboons (genus *Papio*) that had been selectively bred for cholesterolemic response to a cholesterol- and fat-enriched diet, we found progeny with plasma lipoproteins intermediate in size and density between low density lipoproteins (LDL) and HDL (13). These lipoproteins, termed HDL_1, are *1*) induced mainly by feeding a diet rich in cholesterol and saturated fat (14); *2*) rich in cholesteryl esters and apoprotein (apo) A-I; and *3*) heterogeneous (15). As hypothesized for HDL_c (16), HDL_1 may be formed by the accumulation of cholesteryl esters and apoE in HDL_2, except that in the baboon only the largest HDL_1 particles contain apoE.

Recently, subjects with hyperalphalipoproteinemia were reported to have major population of HDL larger than normal HDL_2 (17, 18). Their HDL differed from HDL_c in that it did not inhibit binding of LDL to LDL receptors in cultured human fibroblasts (18). The HDL from these subjects showed a low rate of cholesteryl ester transfer to LDL (17, 18). Since baboon HDL_1 appeared to be similar to the larger HDL that accumulates in the plasma of hyperalphalipoproteinemic subjects (17, 18), we compared the rates of cholesteryl ester transfer among the lipoproteins of high and low HDL_1 baboons.

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HCHF, high cholesterol, high saturated fat; LPDS, lipoprotein-deficient serum; CETP, cholesteryl ester transfer protein.

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MATERIALS AND METHODS

Animals and diets

Adult male and female baboons (genus *Papio*) were selected for high and low HDL_1 levels on the basis of their lipoprotein profiles on a chow diet and on a high cholesterol and high saturated fat (HCHF) diet described in **Table 1**. The animals selected for the high HDL_1 phenotype exhibited large amounts of HDL_1 in their plasma while consuming the HCHF diet and lower amounts while consuming chow. The control animals (low HDL_1 phenotype) had low levels of HDL_1 in plasma on both diets. For this study, the baboons were fed a chow or HCHF diet once a day ad libitum with access to water at all the times. The Southwest Foundation is accredited by the American Association for Accreditation of Laboratory Animal Care and is registered with the U.S. Department of Agriculture. The protocol for this experiment was approved by the institutional Animal Research Committee.

Detection of HDL_1 in plasma

To detect the presence of HDL_1 in plasma, fasting (14–18 h) animals were bled after immobilization with ketamine HCl (10 mg/kg). Blood was collected in tubes containing EDTA (1 mg/ml) and plasma was obtained by low speed centrifugation at 6°C. The plasma was treated with sodium azide (2 mg/dl), chloramphenicol (0.5 mg/dl), gentamycin sulfate (1 mg/dl), and phenylmethyl-sulfonyl fluoride (0.05 mg/dl). For isolation of lipoproteins used in the cholesteryl ester assay, DTNB was added to give a final concentration 1.4 mM in the plasma. The plasma (2 ml) was adjusted to a density of 1.30 g/ml and was layered with solutions of different densities, from 1.21 g/ml to 1.006 g/ml, in SW41 Ti rotor tubes as described earlier (14). The plasma was then ultracentrifuged in an SW41 Ti rotor using a Beckman ultracentrifuge Model L8-70 (Beckman Instruments, Palo Alto, CA) at 39,000 rpm (175,000 *g*) for 24 h at 6°C. After ultracentrifugation, the tube contents were fractionated as described previously (14). Alternatively, the plasma lipoproteins were separated by high performance liquid chromatography (HPLC) using a gel filtration column (a combination of PW 4,000 and PW 3,000) (19). As shown in **Fig. 1**, both density gradient ultracentrifugation and gel filtration methods demonstrated the presence of HDL_1 lipoproteins occurring between LDL and HDL in high HDL_1 animals. Thus these lipoproteins are different from Lp[a], which is larger than LDL (13).

TABLE 1. Compositions of chow and high-cholesterol, high-fat (HCHF) diets

Nutrients	Chow Diet	HCHF Diet[a]
Carbohydrates (% cal)	62	40
Protein (% cal)	28	20
Fat (% cal)	10	40
Energy (kcal per 100 g diet)	329	377
Cholesterol (mg/kcal)	0.03	1.7

[a]HCHF diet was prepared by mixing 81.4% (dry weight basis) of Purina monkey meal 5-5045-6 (a special mix with no added fat, dehydrated alfalfa, sodium chloride, ascorbic acid, or retinyl acetate) with lard (16.5%), sodium chloride (1.1%), retinyl acetate (0.005%), ascorbic acetate (0.2%), and cholesterol (0.74%).

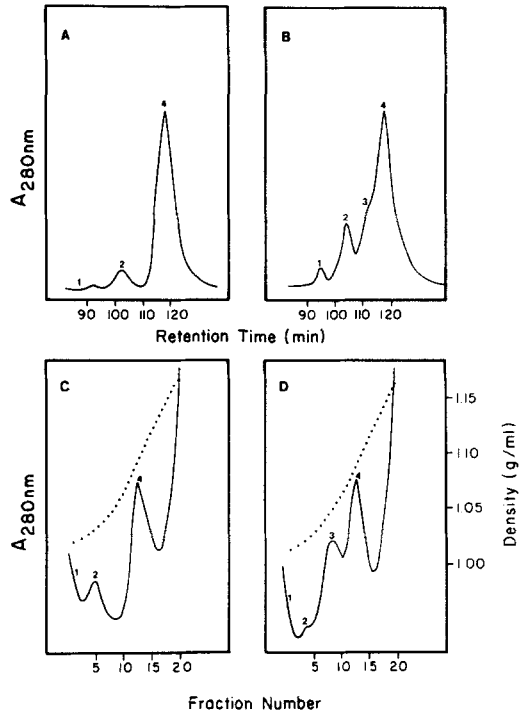

Fig. 1. Plasma lipoprotein patterns of low (A, C) and high (B, D) HDL_1 baboons separated by high performance liquid chromatography using a gel filtration column (A, B) and by density gradient ultracentrifugation (C, D). For HPLC, 0.2 ml of lipoproteins (d <1.21 g/ml) was injected and the absorbance at 280 nm was recorded. For density gradient ultracentrifugation, 2 ml of plasma was adjusted to d 1.30 g/ml by adding solid KBr and layered with various solutions of different densities. After ultracentrifugation, lipoproteins were fractionated and absorbance at 280 nm was recorded. Numbers 1, 2, 3, and 4 correspond to VLDL, LDL, HDL_1, and HDL (HDL_2 + HDL_3), respectively.

Preparation of [^{3}H]cholesteryl ester-labeled HDL and LDL

HDL and HDL_3 from both high and low HDL_1 animals were labeled with [^{3}H]cholesteryl ester by the method of Stein et al. (20). In the first set of experiments, [1,2,6,7- ^{3}H(N)]cholesteryl linoleate (New England Nuclear, Boston, MA) was sonicated (Fisher Scientific Co., Pittsburgh, PA) with Intralipid (Kabi Vitrum Inc., Alameda, CA) diluted in saline. After treatment with 1.4 mM DTNB, plasma lipoproteins (in 10 ml plasma) were labeled by incubation with the Intralipid/[^{3}H]cholesteryl ester mixture for 20 h at 37°C under nitrogen (method 1).

In later experiments, the use of Intralipid was avoided because it could affect the composition of lipoproteins. Tritiated cholesteryl ester (125 μCi) was dissolved in 30 μl of ethanol and added to 6 ml of plasma containing 1.4 mM DTNB and incubated for 20 h at 37°C under nitrogen (method 2). After incubation, the LDL and HDL fractions were isolated by density gradient ultracentrifugation (14). Lipoprotein fractions were dialyzed against saline/EDTA and used as substrates in the cholesteryl ester transfer reaction. In some experiments, HDL_3 (d 1.125–1.21 g/ml) labeled with [^{3}H]cholesteryl ester was prepared by sequential ultracentrifugation in which lipoproteins of d < 1.125 g/ml were first removed. The density of the infranatant was adjusted to 1.21 g/ml by adding solid KBr and was ultracentrifuged for 24 h at a speed of 42,000 rpm (110,000 *g*) at 6°C using a Beckman 50 Ti rotor and an L8-70 ultracentrifuge (Beckman). The HDL_3 was obtained by slicing the top 3-ml layer. The purity of the substrate was checked by ultracentrifugation and greater than 95% of the radioactivity was recovered in the HDL fraction. The substrate was freshly prepared for each experiment.

Assay for cholesteryl ester transfer activity

Cholesteryl ester transfer activity was assayed as described by Koizumi et al. (17). For the first set of experiments, the assay mixture (3 ml) consisted of the entire lipoprotein fraction (d < 1.21 g/ml), [^{3}H]cholesteryl ester-labeled HDL (HDL_3 or HDL_2 + HDL_3) from either high HDL_1 or low HDL_1 animals, and lipoprotein-deficient serum (LPDS) from either high or low HDL_1 animals, in phosphate buffered saline, pH 7.4, with 1.4 mM DTNB to inhibit lecithin:cholesterol acyltransferase. In subsequent experiments, [^{3}H]cholesteryl ester-labeled HDL (125–500 μg of protein) was incubated with VLDL and LDL (equal protein content) in the presence of LPDS from baboon plasma (equivalent to 50–100 μl of plasma) as a source of cholesteryl ester transfer protein (CETP). The incubations were carried out for 1 to 8 h at 4°C (control) or at 37°C and were terminated by placing tubes on ice. The density of the assay mixture was adjusted to 1.045 g/ml by adding solid potassium bromide and overlayered with d 1.045 g/ml potassium bromide solution. The assay mixture was ultracentrifuged in a 50.3 Ti rotor at 45,000 rpm (150,000 *g*) for 22 h at 6°C. The lipoproteins of d < 1.045 g/ml (VLDL + LDL) were obtained by tube slicing. Radioactivity in fractions was counted by mixing 0.5 ml with 10 ml scintillation fluid (Scintisol, Isolab, Akron, OH) and using a liquid scintillation counter (Searle, Nuclear Chicago, Des Plaines, IL).

In all experiments, the difference in transfer at 4°C and 37°C incubations was considered to be due to transfer activity in the LPDS (17). In some cases incubation at 37°C without LPDS was used as control. Values at 4°C with LPDS and 37°C without LPDS were similar. The cholesteryl ester transfer activity was expressed either as percent of total radioactivity or percent of mass transferred. The mass of cholesteryl ester was calculated by dividing the number of counts transferred to VLDL + LDL (after subtracting the counts for 4°C control) by the specific activity of HDL (the average of initial and final specific activity of HDL cholesteryl ester). All experiments were continued for only 4–5 h to avoid isotopic equilibrium.

Analytical methods

HDL_1 and HDL from high HDL_1 baboons and HDL from low HDL_1 baboons were characterized for protein and lipid composition. Protein contents were measured by the method of Lowry et al. (21) using bovine serum albumin as standard. Total cholesterol and triglycerides were measured by using enzymatic kits from Sigma Chemical Co. (St. Louis, MO), and free cholesterol was measured using an Autoflo cholesterol kit (Boehringer Mannheim Diagnostics, Indianapolis, IN) with the modification described earlier (22). Phospholipid phosphorus was measured by the method of Ames and Dubin (23), and the values were multiplied by 25 to estimate phospholipids. HDL delipidation was carried out using 8 volumes acetone–ethanol 2:1 at 4°C followed by ether alone. HDL apoproteins were separated by 10% SDS polyacrylamide slab gel electrophoresis (24).

Statistical analysis

Values in tables are presented as mean ± standard deviation. The values were compared using Student's *t*-test. The data described in Fig. 3 (time-course experiment) were analyzed by analysis of variance using repeated measures.

RESULTS

Composition of HDL_1 and HDL from high and low HDL_1 animals

To compare the composition of HDL_1 with HDL from both high and low HDL_1 animals, plasma from five high and five low HDL_1 animals, maintained on the HCHF diet, was separated by density gradient ultracentrifugation. Fractions corresponding to HDL_1 and HDL (densitites of 1.045–1.07 and 1.07–1.21 g/ml, respectively) were pooled and analyzed for lipid and protein. The compositions of HDL_1 and HDL are given in **Table 2**. The lipid and protein compositions of HDL from both high and low HDL_1 animals were similar. Both HDL and HDL_1 had no detectable apoB. The composition of HDL_1, however, differed considerably from the HDL of both high and low HDL_1 baboons. HDL_1 had significantly ($P < 0.05$) lower protein content and higher cholesterol content than HDL. Cholesteryl esters were the major HDL_1 component. The apoprotein composition of HDL (d 1.063–1.21 g/ml), com-

TABLE 2. Composition of HDL_1 and HDL from low and high HDL_1 animals

Lipoprotein and HDL_1 Phenotype	Number of Animals	Protein	Cholesterol (Unesterified)	Cholesteryl Ester	Triglycerides	Phospholipids
			% of total weight (mean ± SD)			
HDL_1						
Low HDL_1	–	–	–	–	–	–
High HDL_1	5	29.1 ± 5.1[a]	6.9 ± 0.4[b]	33.5 ± 8.5[b]	0.7 ± 0.6	29.8 ± 5.3
HDL						
Low HDL_1	5	46.4 ± 7.0[a]	4.1 ± 0.9[b]	17.4 ± 2.8[b]	1.2 ± 0.2[c]	30.7 ± 3.5
High HDL_1	5	40.9 ± 3.9[a]	4.8 ± 0.5[b]	21.8 ± 0.5[b]	0.8 ± 0.2	31.7 ± 2.7

[a]Protein content of HDL_1 is significantly lower ($P < 0.05$) than that of HDL from either high or low HDL_1 animals.
[b]Values for HDL_1 are significantly higher ($P < 0.05$) than the corresponding values for HDL from either high or low HDL_1 animals.
[c]Triglyceride content of HDL from low HDL_1 animals is significantly higher ($P < 0.05$) than for HDL from high HDL_1 animals.

pared by SDS gel electrophrosis, was not different (**Fig. 2**).

Effect of LPDS on cholesteryl ester transfer from HDL to VLDL and LDL

To determine whether the transfer of cholesteryl ester radioactivity from HDL to VLDL was mediated by CETP, [^{3}H]cholesteryl ester-labeled HDL (from low HDL_1 baboons) was incubated with VLDL + LDL and increasing concentrations of LPDS as a source of CETP. The transfer of cholesterol ester radioactivity from HDL to VLDL + LDL increased linearly with concentrations of LPDS up to 60 μl (equivalent to 60 μl of plasma), beyond which there was no further increase in the transfer rate. Thus, the rate of the transfer reaction was dependent on the LPDS concentration.

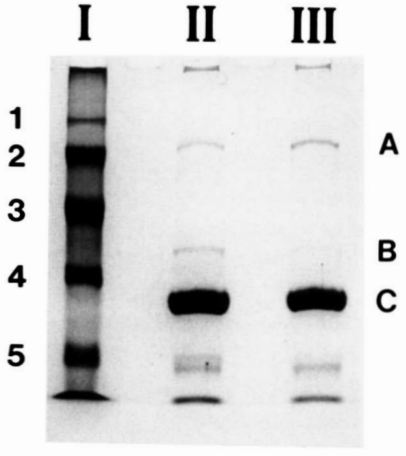

Fig. 2. HDL apoproteins separated by slab gel electrophoresis from a baboon with high HDL_1 (II) and a baboon with low HDL_1 (III). A, B, and C correspond to albumin, apoE, and apoA-I, respectively. The apoproteins were identified by immunoblotting the polyacrylamide gels with antibodies against these apoproteins. Lane (I) shows protein standards with the following molecular weights: 1) phosphorylase b, 94,000; 2) bovine serum albumin, 67,000; 3) ovalbumin, 43,000; 4) carbonic anhydrase, 30,000; and 5) α-lactalbumin, 14,400.

Effect of time on the transfer of cholesteryl ester from HDL_3 to VLDL + LDL in both high and low HDL_1 baboons

To determine the time course of the transfer of cholesteryl ester from HDL_3 to VLDL + LDL in both high and low HDL_1 animals, HDL_3 isolated from plasma of high and low HDL_1 baboons were labeled by method 1. The specific activity of HDL_3 from high HDL_1 baboons was 3.67×10^6 and from low HDL_1 baboons was 3.64×10^6 dpm/mg cholesteryl ester. A similar volume of HDL (171×10^3 dpm for high and 136×10^3 dpm for low) was incubated with 0.3 ml of lipoproteins (d<1.21 g/ml), representing 0.8 ml of plasma in the presence of 0.5 ml of LPDS, derived from 0.57 ml of plasma and 2.1 ml phosphate-buffered saline containing DTNB. The incubations were conducted for 0, 2, 4, 6, and 8 h at 4°C and 37°C. **Fig. 3** shows that the transfer of radioactivity to VLDL + LDL from HDL_3 was strongly affected by the HDL_1 phenotype of the HDL_3 donor. The transfer of cholesteryl ester radioactivity from HDL_3 of high HDL_1 animals was slow, appeared to be linear over the time tested, and did not reach equilibrium. The transfer of cholesteryl ester radioactivity from HDL_3 of low HDL_1 animals to VLDL + LDL was much faster but was also curvilinear over the time tested. At 2 h more radioactive cholesteryl ester was transferred from HDL_3 of low HDL_1 animals than was transferred in 8 h when the HDL_3 substrate was from high HDL_1 animals. The LPDS derived from both low and high HDL_1 animals possessed similar

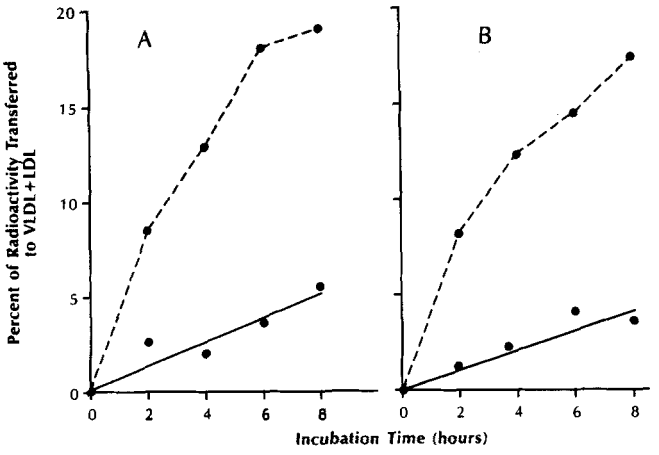

Fig. 3. Transfer of cholesteryl ester radioactivity (% of total) from HDL_3 to VLDL + LDL as a function of time. HDL_3 from the high HDL_1 animals and from two low HDL_1 animals was labeled with [^{3}H]cholesteryl linoleate. The differences in radioactivity transferred to VLDL + LDL at 4°C and 37°C are plotted. A, HDL_3 from high (●—●) and low (●---●) HDL_1 baboons in the presence of LPDS from high HDL_1 baboons. B, HDL_3 from high (●—●) and low (●---●) HDL_1 baboons in presence of LPDS from low HDL_1 baboons. The experiment was conducted with one assay for each time point except at 6 h time point where duplicate assays were performed.

cholesteryl ester transfer activities, whereas the HDL_3 substrate prepared from the two groups of baboons differed dramatically in the rates of cholesteryl ester transfer. Since the LPDS source did not affect cholesteryl ester transfer, the data for HDL_3 from each phenotype with two sources of LPDS were considered as duplicates and analyzed by analysis of variance with repeated measures. Cholesteryl ester transfer from HDL_3 of high HDL_1 baboons was significantly higher ($P = 0.006$) than that from HDL_3 of low HDL_1 baboons. The cholesteryl ester transfer from HDL_3 to VLDL + LDL increased with time ($P = 0.0001$) but this increase was significantly higher ($P = 0.0001$) from HDL_3 of low HDL_1 baboons.

In a similar experiment, where four different animals were used as sources of LPDS, the transfer of cholesteryl ester from HDL_3 of low HDL_1 baboons (21.5 ± 0.8% transfer of radioactivity to VLDL + LDL, n = 4) was significantly higher ($P > 0.01$) than that from HDL_3 of high HDL_1 baboons (4.8 ± 2.7% transfer of radioactivity to VLDL + LDL, n = 4) in 7.25 h.

Cholesteryl ester transfer from HDL to VLDL + LDL of baboons on an HCHF diet

In this experiment three animals with each phenotype were selected for the study of cholesteryl ester transfer from HDL (HDL_2 + HDL_3) to VLDL + LDL. These animals were maintained on the high cholesterol, high saturated fat diet for at least 4 months. HDL (d 1.063–1.21 g/ml) from individual animals was labeled with [^{3}H]cholesteryl linoleate by method 2 as described above (i.e., without Intralipid). The specific activity of HDL from low HDL_1 baboons was 4.15 ± 1.11 × 10^5 dpm and from high HDL_1 baboons was 2.68 ± 0.72 × 10^5 dpm/mg cholesteryl ester. An equal amount of HDL (0.5 mg protein) was used in the incubation. The LPDS (d > 1.21 g/ml) used for incubations was obtained from one baboon with a low HDL_1 phenotype (equivalent to 100 μl of plasma for each incubation). The incubations were carried out for 4 h using VLDL + LDL (0.5 mg protein) from a common donor source. The transfer of cholesteryl ester (both radioactivity and mass) from HDL to VLDL + LDL is shown in **Table 3**. As in the first experiment, the transfer of cholesteryl ester was significantly higher ($P < 0.05$) when the HDL substrate was from low HDL_1 animals than from high HDL_1 animals. The specific activity of cholesteryl ester in VLDL + LDL was approximately one-fourth of that of cholesteryl ester in HDL after the incubation. Thus the isotopic equilibrium was not a factor in these measurements.

Cholesteryl ester transfer from HDL to VLDL + LDL of baboons on a chow diet

To determine whether the different cholesteryl ester transfer rates from HDL to VLDL + LDL noted above were observed only when HDL_3 or HDL was obtained from animals on the HCHF diet, three animals with high HDL_1 and three animals with low HDL_1 were maintained on a chow diet for at least 3 months. HDL (HDL_2 + HDL_3) from these baboons was labeled with [^{3}H]cholesteryl linoleate as described (method 2, without Intralipid). The specific activity of HDL from low HDL_1 baboons was 6.43 ± 3.68 × 10^5 dpm and from high HDL_1 baboons was 5.26 ± 0.53 × 10^5 dpm/mg cholesteryl ester. The assays were carried out as described above using VLDL + LDL and LPDS from a common source. The transfer of cholesteryl esters from HDL to VLDL + LDL is shown in Table 3. The transfer of cholesteryl ester from HDL of low HDL_1 baboons was significantly higher ($P < 0.05$) than that from HDL of high HDL_1 baboons.

TABLE 3. Cholesteryl ester transfer from HDL to VLDL + LDL in baboons fed chow and HCHF diets

	Cholesteryl Ester Transfer	
Diet/Phenotype	Percent of Mass	Percent of Radioactivity
A. Chow diet		
1. High HDL_1	17.1 ± 6.7[a]	16.2 ± 6.3[a]
2. Low HDL_1	29.9 ± 2.8	28.2 ± 3.1
B. HDHF diet		
1. High HDL_1	12.9 ± 7.7[a]	11.7 ± 4.8[a]
2. Low HDL_1	37.1 ± 8.8	33.2 ± 5.9

[a]Values for high HDL_1 baboons are significantly lower ($P < 0.05$) than those for low HDL_1 baboons on both diets.

As in previous experiments, the specific activity of cholesteryl ester in VLDL + LDL was considerably lower than that for cholesteryl ester in HDL after the incubations.

Effect of HDL/(VLDL + LDL) protein ratio of cholesteryl ester transfer

This experiment was conducted to determine whether increase in the HDL/(VLDL + LDL) protein ratio would affect the cholesteryl ester transfer from HDL to VLDL + LDL and whether the differences between high and low HDL_1 baboons were affected by this ratio. For this experiment, HDL from both high and low HDL_1 baboons (fed the HCHF diet) was incubated with VLDL + LDL in the presence of cholesteryl ester transfer protein source (LPDS) for 5 h. The experiment was conducted using three replicates for each assay. The VLDL + LDL protein in these assays varied from one- to sixfold of that of HDL protein added in the assay. The incubation conditions were similar to those described above for the HCHF diet. The cholesteryl ester radioactivity transferred from HDL to VLDL + LDL after 5 h incubation was measured. There was an increase in the percent of HDL radioactivity transferred to VLDL + LDL with the increase in the HDL/VLDL + LDL protein ratio (**Fig. 4**). However, the HDL cholesteryl ester radioactivity transferred to VLDL + LDL was less in high HDL_1 baboons than that in low HDL_1 baboons at each HDL/(VLDL + LDL) protein ratio.

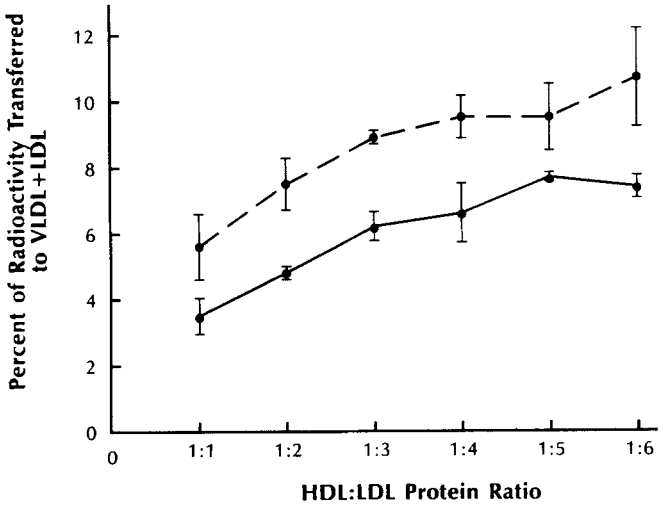

Fig. 4. Effect of HDL/LDL (VLDL + LDL) protein ratio on the transfer of cholesteryl ester radioactivity from HDL to VLDL + LDL mediated by cholesteryl ester transfer activity of the LPDS. [^{3}H]Cholesteryl ester was obtained from low HDL_1 baboons (●---●) or from high HDL_1 baboons (●—●).

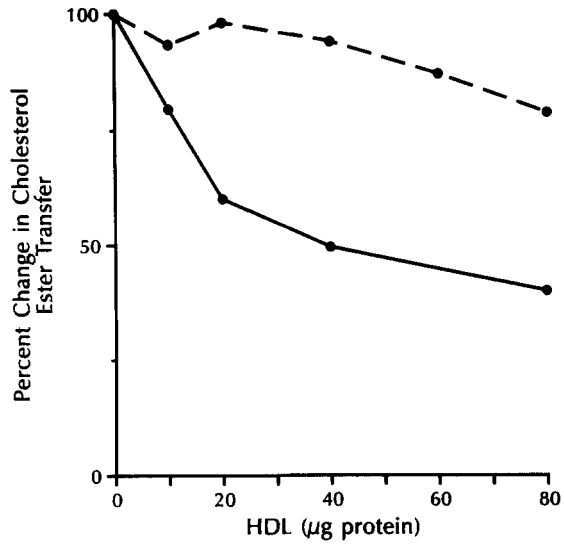

Fig. 5. Effect of added whole HDL from high HDL_1 (●—●) and low HDL_1 (●---●) baboons on the transfer of cholesteryl ester radioactivity from low HDL_1 baboons. The results are expressed as percent change in cholesteryl ester radioactivity transferred to VLDL + LDL. Cholesteryl ester radioactivity transferred from HDL to VLDL + LDL in the experiment without added HDL from high or low HDL_1 baboons was considered as 100% transfer. HDL from low HDL_1 baboons was labeled with [^{3}H]cholesteryl linoleate and was incubated with VLDL + LDL in the presence of LPDS and an increasing amount of HDL from high and low HDL_1 baboons.

Effect of added HDL from high HDL_1 baboons on the transfer of cholesteryl ester radioactivity from HDL of low HDL_1 baboons

This experiment was conducted to determine whether a component of HDL in high HDL_1 baboons may have inhibitory activity against CETP, thus slowing the cholesteryl ester transfer reaction. HDL (125 μg protein) from a low HDL_1 baboon (X4038) was incubated with VLDL + LDL (125 μg protein) and LPDS (equivalent to 100 μl of plasma) in the presence of increasing concentrations of HDL (10, 20, 40, and 80 μg of HDL protein) from a high HDL_1 baboon (X2033) and a low HDL_1 baboon (X4038). The incubations were carried out as described earlier for 4 h. **Fig. 5** describes the results of this experiment. The addition of HDL from the high HDL_1 baboon decreased the CETP-mediated transfer of radioactivity in VLDL + LDL. Cholesteryl ester mass transferred to VLDL + LDL also decreased parallel to cholesteryl ester radioactivity transferred. However, there was very little decrease in the radioactivity (Fig. 5) but no decrease in the mass of cholesterol (data not shown) transferred to VLDL + LDL when HDL from the low HDL_1 baboon was added to the reaction mixture. HDL from the high HDL_1 baboon thus decreased the transfer of cholesteryl ester radioactivity and mass from HDL.

Distribution of inhibitor activity in the plasma

To determine the distribution of inhibitor activity in plasma lipoproteins of high HDL_1 baboons, 2 ml plasma from a high HDL_1 baboon was ultracentrifuged by density gradient ultracentrifugation and 24 fractions were collected as shown in **Fig. 6**. A small amount (0.05 ml) of each fraction was added to a cholesteryl ester transfer assay set up with HDL from a low HDL_1 baboon, and the effect on the transfer of cholesteryl ester was examined. As shown in Fig. 6, fractions 6, 14, and 20 inhibited cholesteryl ester transfer from HDL to VLDL + LDL. These fractions correspond to IDL, HDL, and denser HDL.

Characterization of inhibitor

This experiment was conducted to determine whether the inhibition of CETP activity in the plasma of high HDL_1 baboons was attributable to a protein component of HDL. Delipidated HDL (apoHDL) from a high and a low HDL_1 baboon, after solubilization in 1.0 M Tris buffer (pH 7.4) was added to the reaction mixture in which HDL from a low HDL_1 baboon was incubated with VLDL + LDL in the presence of LPDS (HDL to VLDL + LDL protein ratio was 1:4) as described for the experiment using whole HDL. As a control, a similar amount of protein from a low HDL_1 baboon was added. After 4 h of incubation, the VLDL + LDL fraction was isolated by ultracentrifugation and the radioactivity in both VLDL + LDL and the infranatant fractions was measured. There was no change in radioactivity of cholesteryl ester transferred to VLDL + LDL when delipidated HDL from the low HDL_1 baboon was added. However, the addition of apoHDL from a high HDL_1 baboon at a concentration of 50 and 100 μg decreased the cholesteryl ester transfer by 48% and 70%, respectively.

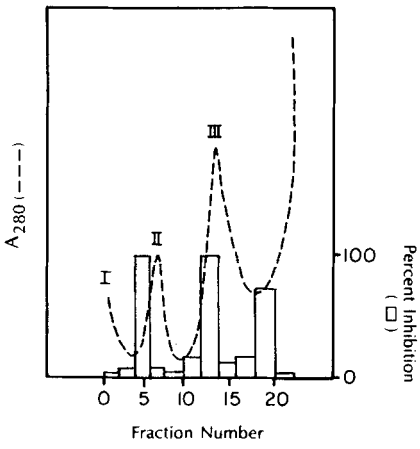

Fig. 6. Effect of added lipoprotein fractions separated by density gradient ultracentrifugation from a baboon with high HDL_1, on the transfer of cholesteryl ester radioactivity from HDL to VLDL + LDL from low HDL_1 baboons. Absorbance at 280 nm (---) shows the lipoprotein pattern and numbers I, II, and III correspond to VLDL, LDL and HDL peaks, respectively. Percent inhibition of cholesteryl ester transfer by each fraction is indicated by bars.

DISCUSSION

Summary of results

This study suggests that some families of baboons that have high levels of HDL on the chow diet and accumulate HDL_1 in their plasma on the HCHF diet have impaired transfer of cholesteryl ester from HDL to lower density lipoproteins. This impairment appears to be attributable to the presence in the HDL of a protein inhibitor of cholesteryl ester transfer. This protein is probably a minor component of HDL protein as described by others (25). The inhibitor is present not only in HDL but also in the IDL fraction.

Observations in other species

Several other species, including rats, pigs, and dogs, have been reported to accumulate HDL_1 in their plasma rather readily. The basis of this accumulation is not fully characterized, although either decreased cholesteryl ester transfer protein or activity or increased activity of a cholesteryl ester transfer protein inhibitor is possible. Kurasawa et al. (26) reported that a homozygous subject with familial hyperalphalipoproteinemia had impaired cholesteryl ester transfer between HDL and LDL. They also reported (18) that the plasma fraction $d > 1.21$ g/ml of the subject had substantial transfer activity with normal HDL, a finding that suggests potentially adequate levels of transfer activity. The HDL from the subject with hyperalphalipoproteinemia was, therefore, suggested to be a poor substrate in the transfer of cholesteryl esters to LDL in the presence of LPDS from normal subjects. The HDL particles that accumulated in this subject, like HDL_1 in baboons, were substantially larger in molecular size than ordinary HDL_2 (26), and their accumulation may have been due to the presence of an inhibitor of cholesteryl ester transfer.

Koizumi et al. (17) reported two hyperalphalipoproteinemic patients with a large HDL that was clearly separated from LDL. The patients' plasma showed no cholesteryl ester transfer, apparently due to a deficiency in transfer activity rather than to abnormal HDL substrate.

Role of CETP in cholesteryl ester transfer

The transfer of cholesteryl ester is catalyzed by protein factors as characterized by several investigators (27–33). Hesler et al. (34) has recently reported that monoclonal antibodies to CETP with a molecular weight of 74,000 neutralize all the cholesteryl ester transfer activities in human plasma, suggesting that a single cholesteryl ester transfer protein is responsible for all transfer activity in the plasma. Both the transfer of cholesteryl esters from

HDL and its particle size are affected by the lipid transfer protein (35). Upon injection of exogenous lipid transfer protein into rats (which are deficient in CETP activity), there is a total loss of apoE-rich HDL_1 particles that predominate in the plasma of rats (36). This loss of larger HDL is associated with an increase in the relative cholesteryl ester content of VLDL and an increase in the triglyceride content of HDL (36). Accumulation of triglyceride-poor and larger HDL due to slower cholesteryl ester transfer reaction in baboons of some families is consistent with these observations in rats (36).

CETP inhibitor

Son and Zilversmit (25) have isolated a plasma protein that inhibits lipid transfer activity. Nishide, Tollefson, and Albers (37) have also reported that an inhibitor of CETP is present on the plasma lipoprotein particles. Our studies also suggest that such an inhibitor protein is present in baboons that may lead to the accumulation of HDL_1 in their plasma. This inhibitor appears to be associated with particles in IDL and HDL regions. Studies are in progress aimed at characterizing the properties of the inhibitor of CETP associated with HDL particles of some families of baboons.

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REFERENCES

1. de Lalla, O. F., and J. W. Gofman. 1954. Ultracentrifugal analysis of serum lipoproteins. *Methods Biochem. Anal.* **1:** 459–478.
2. Patsch, W., G. Schonfeld, A. M. Gotto, Jr., and J. R. Patsch. 1980. Characterization of human high density lipoproteins by zonal ultracentrifugation. *J. Biol. Chem.* **255:** 3178–3185.
3. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nichols. 1981. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **665:** 408–419.
4. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
5. Cheung, M. C., and J. J. Albers. 1977. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J. Clin. Invest.* **60:** 43–50.
6. Schonfeld, G., and B. Pfleger. 1974. The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma as determined by radioimmunoassay. *J. Clin. Invest.* **54:** 236–246.
7. Rifkind, B. M. 1983. Nutrient-high-density lipoprotein relationships: an overview. *Prog. Biochem. Pharmacol.* **19:** 89–109.
8. Shepherd, J., C. J. Packard, J. R. Patsch, and A. M. Gotto, Jr., and O. D. Taunton. 1979. Effects of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein A metabolism. *J. Clin. Invest.* **63:** 858–867.
9. Krauss, R. M., F. T. Lindgren, J. Wingerd, D. D. Bradley, and S. Ramcharan. 1979. Effects of estrogens and progestins on high density lipoproteins. *Lipids.* **14:** 113–118.
10. Schaefer, E. J., D. M. Foster, L. A. Zech, F. T. Lindgren, H. B. Brewer, Jr., and R. I. Levy. 1983. The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. *J. Clin. Endocrinol. Metab.* **57:** 262–267.
11. Thompson, P. D., B. Lazarus, E. Callinane, L. O. Henderson, T. Musliner, R. Eshleman, and P. N. Herbert. 1983. Exercise, diet, or physical characteristics as determinants of HDL-levels in endurance athletes. *Atherosclerosis.* **46:** 333–339.
12. Chang, L. B. F., G. J. Hopkins, and P. J. Barter. 1985. Particle size distribution of high density lipoproteins as a function of plasma triglyceride concentration in human subjects. *Atherosclerosis.* **56:** 61–70.
13. Williams, M. C., R. S. Kushwaha, and H. C. McGill, Jr. 1987. Quantitation of baboon lipoproteins by high performance gel exclusion chromatography. *Lipids.* **22:** 366–374.
14. McGill, H. C., Jr., C. A. McMahan, R. S. Kushwaha, G. E. Mott, and K. D. Carey. 1986. Dietary effects on serum lipoproteins of dyslipoproteinemic baboons with high HDL_1. *Arteriosclerosis.* **6:** 651–663.
15. Babiak, J., E. L. Gong, A. V. Nichols, T. M. Forte, T. J. Kuehl, and H. C. McGill, Jr. 1984. Characterization of HDL and lipoproteins intermediate to LDL and HDL in the serum of pedigreed baboons fed an atherogenic diet. *Atherosclerosis.* **52:** 27–45.
16. Mahley, R. W. 1982. Atherogenic hyperlipoproteinemia. The cellular and molecular biology of plasma lipoproteins altered by dietary fat and cholesterol. *Med. Clin. N. Am.* **66:** 375–402.
17. Koizumi, J., H. Mabuchi, A. Yoshimura, I. Michishita, M. Takeda, H. Itoh, Y. Sakai, T. Sakai, K. Nuda, and R. Takeda. 1985. Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinemia. *Atherosclerosis.* **58:** 175–186.
18. Yokoyama, S., T. Kurasawa, O. Nishikawa, and A. Yamamoto. 1986. High density lipoprotein with poor reactivity to cholesteryl ester transfer reaction observed in a homozygote of familial hyperalphalipoproteinemia. *Artery.* **14:** 43–51.
19. Williams, M. C., J. L. Kelley, and R. S. Kushwaha. 1984. Detection of an abnormal lipoprotein in a large colony of pedigreed baboons using high-performance gel exclusion chromatography. *J. Chromatogr.* **308:** 101–109.
20. Stein, Y., Y. Dabach, G. Hollander, G. Halperin, and O. Stein. 1983. Metabolism of HDL-cholesteryl ester in the rat, studied with a nonhydrolyzable analog, cholesteryl linoleyl ether. *Biochim. Biophys. Acta.* **752:** 98–105.
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
22. Rainwater, D. L., and R. S. Kushwaha. 1988. Characterization of very-low-density lipoproteins isolated from baboons, and fractionated using heparin-Sepharose chromatography. *Biochim. Biophys. Acta.* **958:** 416–423.
23. Ames, B. N., and D. T. Dubin. 1960. The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. *J. Biol. Chem.* **235:** 769–775.
24. Laemmli, U. K. 1970. Cleavage of structural proteins during

the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
25. Son. Y-S., C., and D. B. Zilversmit. 1984. Purification and characterization of human plasma proteins that inhibit lipid transfer activities. *Biochim. Biophys. Acta.* **795:** 473–480.
26. Kurasawa, T., S. Yokoyama, Y. Miyake, T. Yamamura, and A. Yamamoto. 1985. Rate of cholesterol ester transfer between high and low density lipoproteins in human serum and a case with decreased transfer rate in association with hyperalphalipoproteinemia. *J. Biochem.* **98:** 1499–1508.
27. Morton, R. E., and D. B. Zilversmit. 1982. Purification and characterization of lipid transfer protein(s) from human lipoprotein-deficient plasma. *J. Lipid Res.* **23:** 1058–1067.
28. Ihm, J., J. L. Ellsworth, B. Chataing, and J. A. K. Harmony. 1982. Plasma protein-facilitated coupled exchange of phosphatidylcholine and cholesteryl ester in the absence of cholesterol esterification. *J. Biol. Chem.* **257:** 4818–4827.
29. Tall, A. R., E. Abreu, and J. Shuman. 1983. Separation of a plasma phospholipid transfer protein from cholesterol ester/phospholipid exchange protein. *J. Biol. Chem.* **258:** 2174–2180.
30. Chajek, T., and C. J. Fielding. 1978. Isolation and characterization of a human serum cholesteryl ester transfer protein. *Proc. Natl. Acad. Sci. USA.* **75:** 3445–3449.
31. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27:** 361–367.
32. Granot, E., I. Tabas, and A. R. Tall. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured HepG2 cells. *J. Biol. Chem.* **262:** 3482–3487.
33. Albers, J. J., J. H. Tollefson, C. H. Chen, and A. Steinmetz. 1984. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis.* **4:** 49–58.
34. Hesler, C. B., A. R. Tall, T. L. Swenson, P. K. Weech, Y. L. Marcel, and R. W. Milne. 1988. Monoclonal antibodies to the M_r 74,000 cholesteryl ester transfer protein neutralize all of the cholesteryl ester and triglyceride transfer activities in human plasma. *J. Biol. Chem.* **263:** 5020–5023.
35. Barter, P. J., G. J. Hopkins, and Y. C. Ha. 1987. The role of lipid transfer proteins in plasma lipoprotein metabolism. *Am. Heart J.* **113:** 538–542.
36. Ha, Y. C., L. B. F. Chang, and P. J. Barter. 1985. Effects of injecting exogenous lipid transfer protein into rats. *Biochim. Biophys. Acta.* **833:** 203–210.
37. Nishide, T., J. H. Tollefson, and J. J. Albers. 1989. Inhibition of lipid transfer by a unique high density lipoprotein subclass containing an inhibitor protein. *J. Lipid Res.* **30:** 149–158.