Intravascular metabolism of lipoprotein cholesteryl esters in African green monkeys: differential fate of doubly labeled cholesteryl oleate

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Abstract High density lipoproteins (HDL), doubly labeled with [^{3}H]cholesteryl oleate and cholesteryl [^{14}C]oleate, were reinjected to study HDL cholesteryl ester metabolism in African green monkeys. The transfer of labeled HDL cholesteryl ester to low density lipoprotein (LDL) was rapid and equilibration of the [^{3}H]cholesteryl oleate and cholesteryl [^{14}C]oleate specific activities in LDL and HDL occurred within 90 min after reinjection. The apparent rates of disappearance from the circulation of the two moieties of the cholesteryl ester were different. In the same four animals, the residence time for the turnover of plasma [^{3}H]cholesterol averaged 6.1 days while the residence time for the removal of cholesteryl [^{14}C]oleate from plasma was approximately 2.1 days. These results suggest that for some lipoprotein cholesteryl esters removed from plasma, the cholesterol moiety subsequently reappeared in plasma. The difference between the rate of decay of the ^{14}C-labeled fatty acid moiety, which represents all of the cholesteryl ester removed from plasma (0.48 pools/day) and the decay of the ^{3}H-labeled cholesterol moiety, which represents the sum of cholesteryl ester removal and cholesterol reappearance (0.16 pools/day), is the fraction of the cholesteryl ester pool recycled per day (0.32 pools/day or 22.5 mg/kg per day). In other words, approximately 68% of the cholesterol moiety that was removed from plasma as cholesteryl oleate reappeared in the plasma cholesterol pool. These studies support the concept that an efficient reutilization cycle for plasma cholesterol occurs, i.e., the cholesteryl ester molecule can exit and the cholesterol moiety can re-enter plasma without effective equilibration of the cholesterol moiety with extravascular cholesterol pools. —**Thomas, M. S., and L. L. Rudel.** Intravascular metabolism of lipoprotein cholesteryl esters in African green monkeys: differential fate of doubly labeled cholesteryl oleate. *J. Lipid Res.* 1987. **28:** 572–581.

Supplementary key words nonhuman primates • low density lipoproteins • high density lipoproteins • lecithin:cholesterol acyltransferase • plasma turnover • fractional catabolic rate • cholesterol

The African green monkey has been shown to be an excellent model for study of cholesterol metabolism. Since elevated dietary cholesterol levels have been shown to induce atherosclerosis in this primate (1), numerous aspects of dietary cholesterol metabolism have been studied. Sterol balance studies have been carried out to define the dynamics of whole body cholesterol metabolism in this species (2). The characteristics of cholesterol absorption by the intestine have been defined with a thoracic lymph duct fistula preparation (3), and aspects of cholesterol secretion in lipoproteins by the isolated, perfused liver have also been studied (4, 5). This species is known to develop cholesterol gallstones when fed a high fat, cholesterol-containing diet (6), and formation of gallstones is exacerbated by substitution of polyunsaturated fat for saturated fat in the diet (7, 8). The plasma lipoprotein response to dietary cholesterol has been shown to closely mimic the pattern seen in type II hyperlipoproteinemia in human beings (9). Substitution of polyunsaturated fat for saturated fat in the diet has been shown to lower LDL and HDL concentrations (10) in a manner similar to that described for human beings (11).

In spite of the comprehensive nature of the studies on cholesterol metabolism in this species, much remains to be learned. Intravascular metabolism of cholesterol appears to be similar to that of human beings, with the presence of an active cholesteryl ester transfer system (12) similar to that described in man (13, 14). Humans have a well-characterized lecithin:cholesterol acyl transferase (LCAT) enzyme (15, 16) and the LCAT levels in monkeys appear similar and are sensitive to dietary perturbations (17). We have found that the liver of this species produces discoidal HDL precursors of spherical, plasma HDL (4) typical of humans with LCAT deficiency (18). These discs

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; HPLC, high performance liquid chromatography; FCR, fractional clearance rate; PR, production rate; TLC, thin-layer chromatography.

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rapidly become spherical, cholesteryl ester-enriched HDL upon incubation in vitro with purified LCAT (19). Therefore, study of the metabolism of HDL cholesteryl esters in the plasma of these animals promises to provide information on cholesteryl ester movement in plasma apropos to the human situation.

Studies on the fate of plasma cholesteryl esters in humans have been carried out (20–22), however the mechanisms of cholesteryl ester metabolism have not been determined. Given the apparent similarities in lipoprotein cholesterol metabolism between human beings and African green monkeys, we have initiated studies of cholesteryl ester turnover in vivo in the primate model. A recent study in rhesus monkeys has demonstrated that cholesteryl esters are in rapid equilibrium among lipoproteins and that radioactive free cholesterol appears in the plasma soon after reinjection of cholesteryl ester-labeled lipoproteins (23). In the present work, studies were performed in which HDL cholesteryl esters were doubly labeled in vitro with [^{3}H]cholesteryl oleate and cholesteryl [^{14}C]oleate and then reinjected so that the in vivo fate of both labeled moieties could be determined. Interestingly, the cholesterol moiety of the doubly labeled cholesteryl ester did not have the same apparent rate of disappearance from plasma as the oleate moiety of the cholesteryl ester. Rather, the former had a slower fractional clearance rate (FCR).

METHODS

Animals and reinjection study protocol

The animals used for these studies were adult male African green monkeys (*Cercopithecus aethiops*) of the vervet subspecies, weighing 3.5–4.5 kg (n = 10). The monkeys used in these studies were available from an ongoing experiment and, for at least 2 years prior to the present studies, were fed a semipurified diet containing 0.8 mg/kcal cholesterol with 40% of calories as saturated fat (P/S = 0.3). For at least 14 days prior to a turnover study, all animals were adapted to wearing monkey jackets equipped with tethers (Alice King Chatham Industries, Los Angeles, CA).

For turnover studies, heparinized silastic tubing (0.04 I.D. × 0.085 inch O.D.) was surgically implanted into both the femoral vein and artery of each of the monkeys to be used in a study as described previously (24). To allow complete recovery from the effects of surgery, reinjection studies were usually done 4–5 days after cannulation. Recipient animals were fasted overnight before injection at about 9 AM to begin the study. Fasting was continued for the first 6 hr of the study and thereafter, animals were fed at their normally scheduled times of 2:30 PM and 9:00 AM. The radiolabeled lipoprotein dose was injected into the femoral vein and blood samples (3 ml) were withdrawn from the arterial line. Blood was collected in precooled (0–4°C) tubes containing a final concentration of 1 mg/ml ethylenediaminetetraacetic acid (EDTA), 0.5 mg/ml sodium azide (NaN_3), and 0.4 mg/ml 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), pH 7.4. Total plasma cholesterol was measured in five animals according to the method of Rudel and Morris (25), in approximately 15 samples collected throughout 5 days of the metabolic study. The coefficient of variation of the total plasma cholesterol was 4.3%.

Preparation and purification of lipoproteins containing radiolabeled cholesteryl esters

[1,2,6,7-^{3}H(N)]cholesteryl oleate (82.7 Ci/mmol) and cholesteryl [1-^{14}C]oleate (56.6 mCi/mmol) were purchased from New England Nuclear (Boston, MA). Purification of the labeled cholesteryl esters was accomplished by reverse phase high performance liquid chromatography (HPLC) using the method of Carroll and Rudel (26). In all samples used, purity was greater than 97%.

HDL were either singly labeled ([^{3}H]cholesteryl oleate, n = 2), or doubly labeled ([^{3}H]cholesteryl [^{14}C]oleate, n = 4) in vitro by the method described previously (12). For singly labeled lipoproteins, 0.5–1.0 mCi of [^{3}H]cholesteryl oleate, and for doubly labeled lipoproteins 500 μCi of [^{3}H]cholesteryl oleate and 125 μCi of cholesteryl[^{14}C]oleate dissolved in chloroform were added to a 10-ml acid-washed glass tube. The carrier solvent was gradually evaporated under a stream of nitrogen and the radiolabel was evenly coated onto the sides of the tube. Eight to ten ml of freshly isolated plasma (containing a final concentration of 1.4 mM DTNB) was added and the tube was incubated at 37°C for 5 hr in a shaking water bath. After incubation the plasma was cooled to 4°C and the HDL was purified by a combination of ultracentrifugation and agarose column chromatography as described previously (27). The purified, labeled HDL eluted from the column was pooled and concentrated by dialysis at 4°C against dry Dextran T-500 (Pharmacia Fine Chemicals, Piscataway, NJ) to a volume of approximately 5 ml. It was then dialyzed against 0.9% NaCl containing 0.01% EDTA. Immediately prior to injection, the labeled HDL was filtered (0.22 μm, Millex-GS) and an exact volume (4.0 ml) was removed for injection. Each animal received approximately 8.0–9.0 μCi of ^{3}H radioactivity for single label experiments, and 4.5 μCi of ^{3}H radioactivity and 0.95 μCi of ^{14}C radioactivity for double label experiments, in approximately 7 mg of autologous total HDL cholesteryl ester. Plasma isolation was begun at 4°C immediately after blood collection as described previously (12).

The preparation of labeled LDL for injection studies was carried out using plasma incubation for cholesteryl

ester labeling by exchange, ultracentrifugation, and agarose column chromatography as described for the HDL preparations and 1.0 μCi of [^{3}H]cholesteryl oleate was injected in 7.7 mg of LDL cholesteryl ester. In studies in which total plasma lipoproteins were reinjected, the d < 1.225 g/ml fraction from the initial ultracentrifugation of whole plasma (27) was taken and dialyzed against 0.9% NaCl containing 0.1% EDTA and 0.02% azide. In these experiments 6.3 μCi of ^{3}H radioactivity and 1.9 μCi of ^{14}C radioactivity were reinjected in a total of 15.1 mg of plasma lipoprotein cholesteryl ester.

Rapid isolation of plasma lipoproteins

The method of Chung et al. (28) was used for the rapid isolation of lipoproteins for studies in which the kinetics of transfer of labeled cholesteryl esters among lipoproteins was measured. Solid KBr (0.490 g/ml plasma) was added to adjust the density of 1.0 ml of plasma to 1.30 g/ml. The density-adjusted plasma was placed in a 5-ml polyallomer tube and overlayered with a solution of 0.9% NaCl containing 0.01% EDTA and 0.02% NaN_3. Tubes were loaded into a Beckman vertical VTi65 rotor and centrifuged at 5°C for 75 min. at 50,000 rpm. The centrifuge was then stopped with the brake on. Fluorinert® was forced into the tube through the bottom and the sample was drained from the top of the tube directly into a fraction collector set to collect 0.25 ml fractions. Aliquots (40 μl) were taken from each fraction for radioactivity measurements to assess the relative position of the LDL and HDL peaks. In some cases, the position of the lipoproteins in the gradient was also determined by running agarose gel electrophoresis (29) on each gradient fraction and the mass distribution of lipoprotein cholesterol across the gradient was found to be similar to the distribution of radioactivity.

Lipid extraction and thin-layer chromatography

Total lipid extracts of the lipoprotein fractions were prepared by extraction with chloroform–methanol 2:1 (v/v) containing 0.01% (w/v) butylated hydroxytoluene (BHT) (30). Lipid classes were separated by thin-layer chromatography (TLC) on silica gel 60F-254 plates (Brinkmann Instruments, Inc., Westbury, NY) with hexane–diethyl ether–glacial acetic acid 80:20:1 as the developing solvent. The location of the labeled cholesteryl ester was verified by running a cholesteryl ester standard (Nu-Chek-Prep Inc., Elysian, MN) with each TLC plate. Areas corresponding to free cholesterol and cholesteryl ester were scraped from the plate, eluted from the silica gel using chloroform, and the mass of cholesterol was quantitated by the method of Rudel and Morris (25). Recovery of cholesterol from TLC plates was between 80 and 95%. After evaporating an aliquot of the eluate in a 7-ml vial, the amount of radioactivity in these fractions was measured in 4 ml of Aquasol-2 (New England Nuclear) with a Beckman LS 7000 scintillation counter programmed for double-isotope counting with automatic-quench correction.

Separation of cholesteryl ester using high performance liquid chromatography

Lipoprotein cholesteryl esters were separated into their individual classes by reverse phase-HPLC essentially as described by Carroll and Rudel (26), with minor modifications. The water gradient was omitted and was replaced with an isocratic acetonitrile–tetrahydrofuran 70:30 (v/v) (Fisher Chemical Co., Fairlawn, NJ) mobile phase. Cholesteryl heptadecanoate (80 μg) was added as an internal standard to sample aliquots containing 86–110 μg of lipoprotein cholesteryl ester. The samples were filtered using an Acrodisc-CR 0.45-μm filter (Gelman Sciences, Inc., Ann Arbor, MI) and were concentrated by evaporating the extraction solvent under nitrogen. Samples were then redissolved in 30 μl of tetrahydrofuran–acetonitrile 80:20 (v/v) and injected onto the reverse-phase column (25 cm × 4.6 mm, Altex 10-μ Ultrasphere ODS, Rainin Instruments, Woburn, MA). The HPLC analyses were carried out using a Dupont Model 850 liquid chromatograph. The mass of cholesteryl ester in the samples was calculated from standard curves of mass versus peak area and was corrected for loss based on recovery of the internal standard (26). Radioactivity measurements on the HPLC eluate were automatically and continuously monitored for ^{3}H and ^{14}C with a continuous flow scintillation counter (Flo-One HP, Radiomatic Instruments and Chemical Co., Tampa, FL). Budget-Solve (Research Products International Corp., Elk Grove Village, IL) was the scintillation fluid used in these analyses. The Flo-One ^{3}H and ^{14}C windows were set using standard calibration procedures. The counting efficiency for each of the windows was; 9% for the ^{3}H window, and 57% for the ^{14}C window, with an 11% spill of the ^{14}C into the ^{3}H window.

Calculations

Plots of percent of radioactivity remaining in plasma at 5 min versus time were analyzed by "curve peeling" as described by Matthews (31) and were found to fit a two-pool model. Each line representing an individual pool was evaluated for the goodness of fit by use of a correlation coefficient. In each case, the correlation coefficient was 0.988 or greater. The terminal turnover curve of the first exponent was subject to log-linear regression analysis. The best fit line was extrapolated back to time zero with the slope of the line commonly referred to as b and the y-intercept called B. Ln 2/b is equal to the half-life ($t_{1/2}$b) of the more slowly disappearing radioactivity. Data points from the first exponential (early time points) were then subtracted from the extrapolated line and plotted as a function of time. The slope a and y-intercept A of the newly created line were determined by log-linear regres-

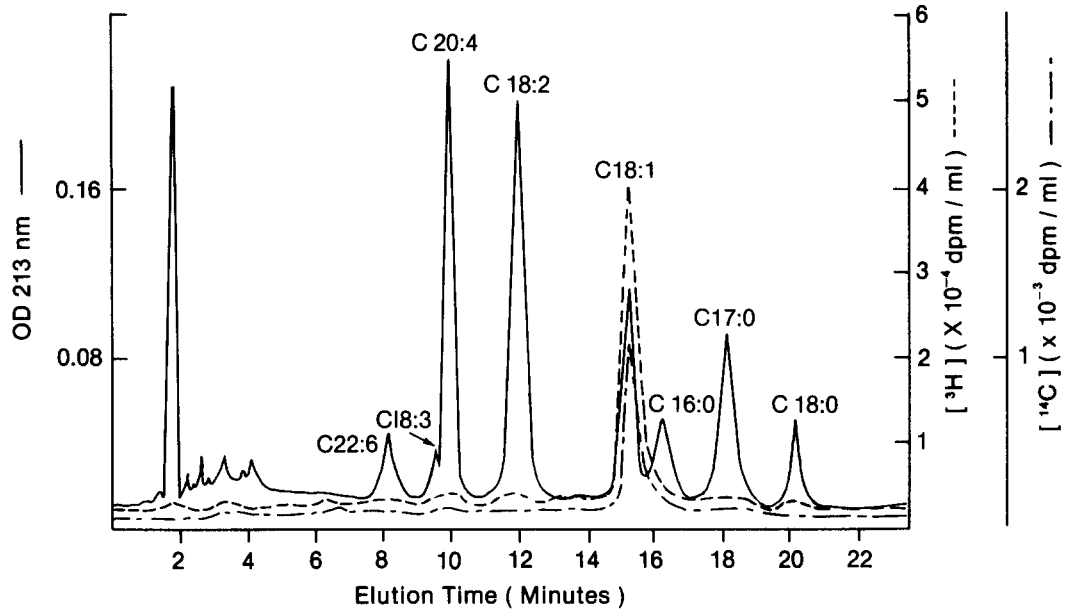

Fig. 1. High performance liquid chromatographic separation of in vitro labeled HDL cholesteryl esters from animal #259. Cholesteryl esters were isolated from TLC plates and approximately 250 μg of esterified cholesterol was applied to a reverse-phase ODS column. Peak identification: C22:6, cholesteryl docosahexaenoate; C18:3, cholesteryl linolenate; C20:4, cholesteryl arachidonate; C18:2, cholesteryl linoleate; C18:1, cholesteryl oleate; C16:0, cholesteryl palmitate; C18:0, cholesteryl stearate. The molar percent distribution for this animal was: C18:3, <1%; C20:4, 10.7%; C18:2, 32.1%; C18:1, 38.7%; C16:0, 12.6%; C18:0, 5.6%.

sion analysis. Ln2/a is equal to the half-life ($t_{1/2}a$) of the more rapidly disappearing radioactivity. Fractional catabolic rates (FCR) were calculated as $100(A/a + B/b)^{-1}$ and the production rates (PR_a) were calculated as the FCR times the total plasma cholesterol pool size. Residence time was calculated as 1/FCR.

For each monkey the plasma volume was assumed to be 3.5% of body weight. In two animals, the plasma volume was determined by injecting a known amount of Evans blue dye (32). The values for plasma volume for both of the animals were found to be within 5% of the 3.5% of body weight estimate. In order to compare data among the animals, the radioactivity remaining in the 5-min plasma sample for each individual animal was taken to represent 100%.

RESULTS

Fig. 1 shows a chromatogram of the HPLC separation of cholesteryl esters isolated prior to reinjection from doubly labeled HDL. These analyses indicated that 97.7 ± 0.2% (mean ± SD, n = 4) of the ^3H and ^{14}C was associated with the cholesteryl oleate peak; no radioactivity was found in the region of free cholesterol (about 4 min), or from the free cholesterol region of the TLC plate (data not shown).

After reinjection of doubly labeled autologous HDL into fasting African green monkeys, the LDL and HDL [^{3}H]cholesteryl oleate specific activity was found to equilibrate within 60 to 90 min, as shown in **Fig. 2.** Similar to the data in Fig. 2 the LDL and HDL cholesteryl [^{14}C]oleate specific activities were also found to equilibrate within 90 min (data not shown). In view of

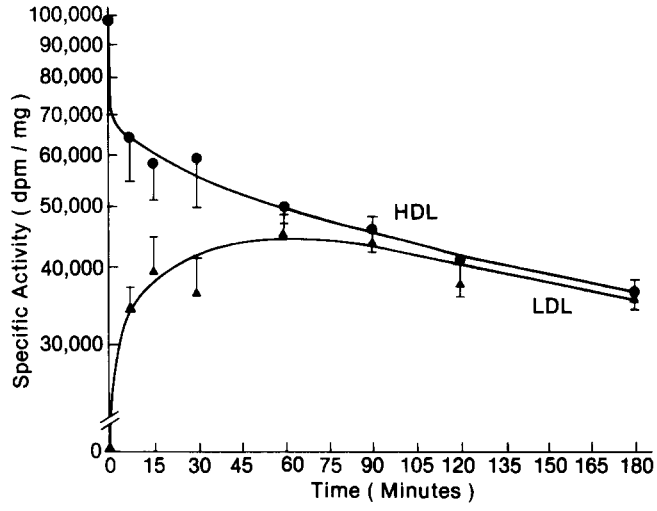

Fig. 2. Equilibration of HDL and LDL [^{3}H]cholesteryl oleate specific activity in vivo. Following reinjection of labeled HDL, plasma lipoproteins were rapidly separated at the indicated points by density gradient centrifugation to obtain LDL and HDL as described in Methods. The [^{3}H]cholesteryl oleate specific activities of LDL and HDL were then measured as described in Methods. Each point represents mean ± SD of four experiments.

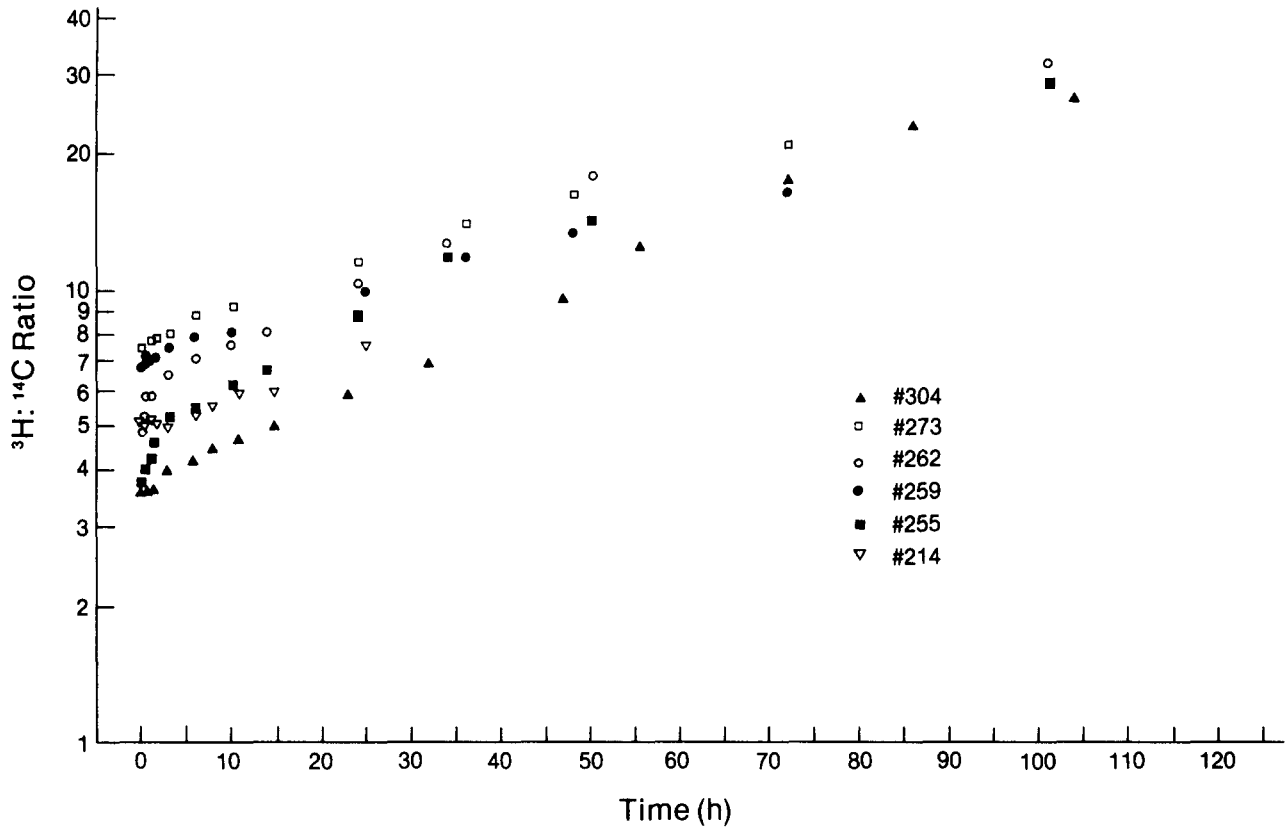

Fig. 3. Time-dependent plasma ^{3}H:^{14}C ratio increase after reinjection of lipoproteins labeled with [^{3}H]cholesteryl [^{14}C]oleate. The ratio of ^{3}H:^{14}C in whole plasma was measured at various times throughout the experiment. Each symbol represents the data from a different animal after the reinjection of an individually prepared doubly labeled lipoprotein dose. The identity of the doubly labeled lipoprotein preparation for each of the animals was: #304, d < 1.225 g/ml plasma lipoproteins; #273, HDL; #262, HDL; #259, HDL; #255, HDL; #214, d < 1.225 g/ml plasma lipoproteins.

this rapid equilibration between LDL and HDL cholesteryl esters following reinjection, subsequent data are presented as total plasma radioactivity.

The analysis of plasma ^{3}H and ^{14}C radioactivity over the course of a 4.5-day metabolic study revealed a time-related increase in the ratio of ^{3}H:^{14}C, as indicated in **Fig. 3.** This figure shows a log-linear relationship between the ^{3}H:^{14}C ratio with time for each of the six animals studied. The average of these ratios changed from 6.3 ± 1.5 to 9.6 ± 2.6 (mean ± SD, n = 6) after 24 hr in the circulation. The form of doubly labeled lipoprotein injected (HDL, LDL, or d > 1.225 g/ml lipoproteins) did not change this outcome.

Plots of the disappearance rate of the ^{3}H and ^{14}C radioactivity from plasma after HDL reinjection are presented in **Fig. 4.** Data for four animals have been averaged together. Each data point represents the average of n = 2 or n =4 plasma time samples. The curves demonstrate a much more rapid disappearance of ^{14}C radioactivity than of the ^{3}H radioactivity. Fractional catabolic rates were derived from the analysis of the log-linear disappearance of plasma ^{14}C and ^{3}H radioactivity for each individual animal and are listed in **Table 1.** The average FCR for the ^{3}H- and ^{14}C-labeled moieties were 0.16 and 0.48 pools/day, respectively.

A comparison of FCR versus plasma cholesterol pool size was made in eleven separate animals as shown in **Fig. 5.** The FCRs used in this figure were calculated for 1 day of decay from plasma since this allowed comparison of a larger number of animals. In several experiments, only enough ^{14}C radioactivity was injected to accurately monitor its disappearance for 24 hr. The relative difference among animals did not change with this method even though the absolute values for FCR were uniformly higher when only 1 day of decay was used. Negative correlations were found between the FCR and the cholesterol pool size for both cholesteryl ester moieties. The data for [^{3}H]cholesteryl oleate had a correlation coefficient of $r = -0.73$ ($P < 0.01$), while the data for cholesteryl [^{14}C]oleate had a correlation coefficient of $r = -0.78$ ($P < 0.05$). Thus, the larger the plasma cholesterol pool size, the more slowly the labeled cholesteryl ester disappeared from plasma. The difference between the turnover rates of the [^{3}H]cholesterol and [^{14}C]oleate was

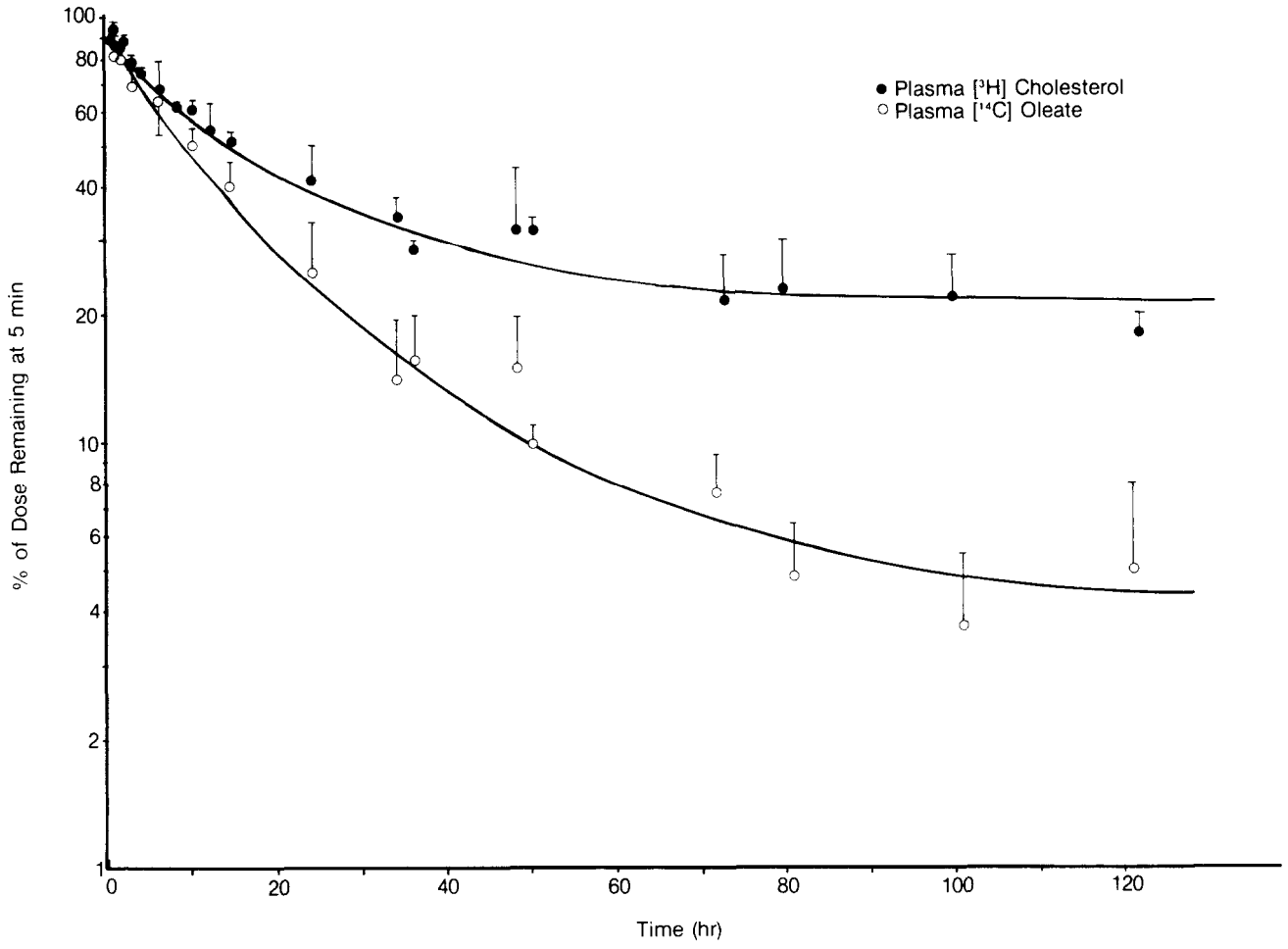

Fig. 4. Total plasma disappearance of radiolabeled cholesteryl esters. After the reinjection of [^{3}H]cholesteryl [^{14}C]oleate-labeled HDL, the total plasma ^{3}H (○) and ^{14}C (●) radioactivity was measured at various times throughout the experiment. These measurements are expressed as the percent of injected dose remaining at 5 min for ^{3}H and ^{14}C radioactivity plotted against time. ^{3}H and ^{14}C radioactivity concentrations from four animals were normalized before averaging. Each point represents the mean ± SD of two or four observations. The averaged percentage of ^{3}H and ^{14}C radioactivity originally injected remaining in plasma at 5 min was 72.3% and 73.6%, respectively.

largest when pool sizes were smallest and FCRs were highest. All data points fit the same lines regardless of the vehicle for injection into the animal.

In several studies aliquots of whole plasma were subjected to lipid extraction, and these extracts were analyzed by TLC to obtain the free to esterified cholesterol radioactivity distribution over the 4.5-day period. **Fig. 6** shows that unesterified [^{3}H]cholesterol in plasma increased with time until the percentage of total ^{3}H radioactivity as free cholesterol reached 25% between 15–20 hr after injection. After equilibration, the percentage distribution of [^{3}H]cholesterol as free versus ester cholesterol corresponded to the mass distribution of these moieties (data not shown). In contrast to these data, the redistribution of [^{14}C]oleate from cholesteryl [^{14}C]oleate into other lipid classes separated by TLC was not detected. Furthermore, analyses by HPLC showed that all plasma cholesteryl esters became labeled with [^{3}H]cholesterol with time as shown in **Table 2.** Over the 48 hr studied the percent distribution of [^{3}H]cholesteryl oleate decreased while the percent of ^{3}H radioactivity in the other plasma cholesteryl esters increased.

TABLE 1. Fractional catabolic rates (FCR) based on 4.5-day plasma cholesterol clearance in African green monkeys

Animal Number	HDL Labeled Moiety		Plasma Cholesterol Pool Size
	[^{3}H]Cholesterol	[^{14}C]Oleate	
	plasma FCR (day^{-1})		*mg/kg*
259	0.18	0.37	94.5
255	0.19	0.68	87.5
262	0.15	0.51	96.9
273	0.14	0.36	115.7
Mean ± SD	0.16 ± 0.02	0.48 ± 0.15	98.6 ± 12.0

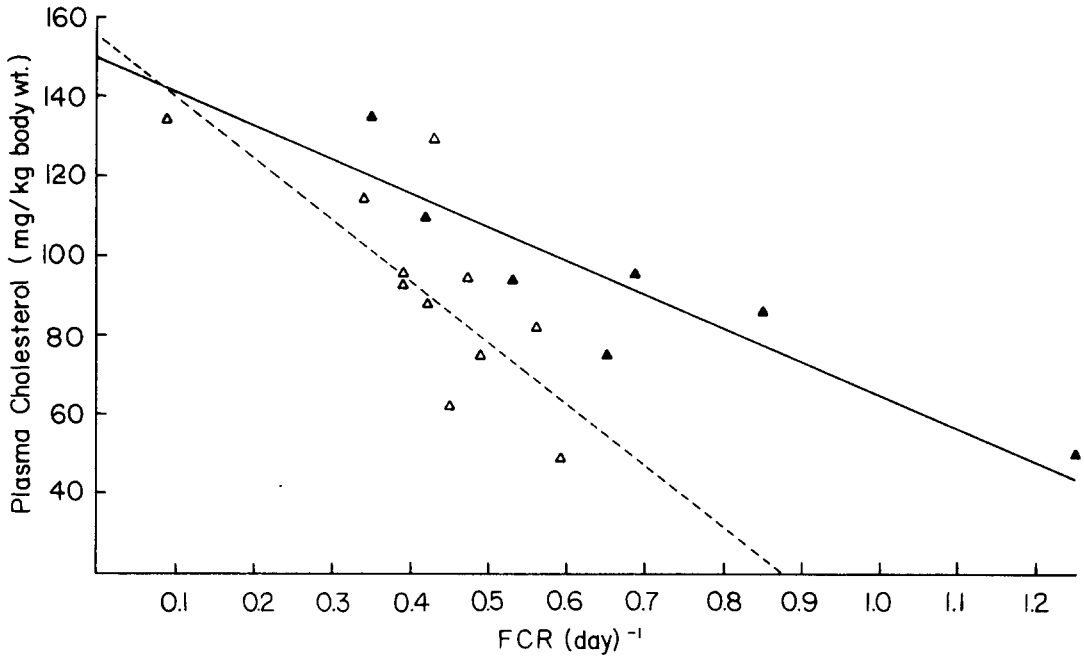

Fig. 5. Relationships between the fractional catabolic rate of lipoprotein [^{3}H]cholesteryl [^{14}C]oleate and the total plasma cholesterol pool size. The FCR were derived during the first 24 hr of decay from plasma of lipoproteins labeled with [^{3}H]cholesteryl oleate (△--△), and cholesteryl [^{14}C]oleate (▲—▲). Each point represents the data from an individual animal. Data from injections of cholesteryl ester-labeled HDL, LDL, and whole plasma have all been plotted with no apparent difference due to source of labeled cholesteryl ester.

DISCUSSION

Doubly labeled cholesteryl esters in lipoproteins were injected into African green monkeys for the purpose of studying the metabolism of cholesteryl ester molecules in vivo. Our original intent was to study the metabolism of HDL cholesteryl esters per se, but it soon became apparent that cholesteryl esters of HDL were in rapid exchange equilibrium with those of the other plasma lipoproteins (Fig. 2) and the decay rate of cholesteryl ester from the plasma was independent of the lipoprotein fraction in which it was injected (Figs. 4 and 5). This outcome demonstrated that plasma cholesteryl esters represent essentially a single or common pool in these primates. An unexpected finding was that the turnover rate of the fatty acid moiety of cholesteryl esters from this pool was apparently more rapid than the cholesterol moiety of the cholesteryl ester. In fact, the FCR for the cholesteryl [^{14}C]oleate (0.48 day^{-1}) was 3 times faster than the FCR for the [^{3}H]cholesteryl oleate (0.16 day^{-1}), see Table 1. Our interpretation of these findings is that the faster clearance rate must more closely approximate the actual rate of cholesteryl ester clearance from plasma, while the slower clearance rate for the cholesterol moiety probably reflects some form of reutilization in addition to clearance.

The fractional catabolic rates for cholesteryl esters can be compared to those of 0.36 day^{-1} for apoA-I (24) and 0.72 day^{-1} for apoB (33) that have been determined in other African green monkeys receiving similar diets. The fact that the fractional clearance rate for cholesteryl ester removed from plasma is slightly faster than for apoA-I and slower than for apoB is likely due to the exchangeability of cholesteryl esters between lipoprotein particles. Cholesteryl ester exit from plasma does not appear to be due to the clearance of only one particular lipoprotein particle. For example, if the FCR for cholesteryl ester was the same as for apoB, it would suggest that clearance of the LDL particle was the primary means for cholesteryl ester removal from plasma. Rather, it appears that some combination of clearance mechanisms occurs, perhaps including removal of whole LDL particles, HDL particles, and even VLDL particles and individual cholesteryl esters. The fact that the fractional clearance rate of cholesteryl esters is inversely proportional to plasma cholesterol pool size (Fig. 5) is consistent with the possibility that the removal process, presumably receptor-mediated lipoprotein clearance, is rate-limiting thereby resulting in the increased plasma pool of cholesterol.

For injected cholesteryl esters, the relative efficiency of reutilization of the labeled cholesterol but not the labeled fatty acid moiety suggests that cholesteryl ester hydrolysis must be a key component in the pathway of plasma cholesteryl ester metabolism. Little evidence for cholesteryl ester hydrolysis in the plasma compartment of humans and nonhuman primates is available. One recent study with the rhesus monkey plasma by Goldberg, et al. (23)

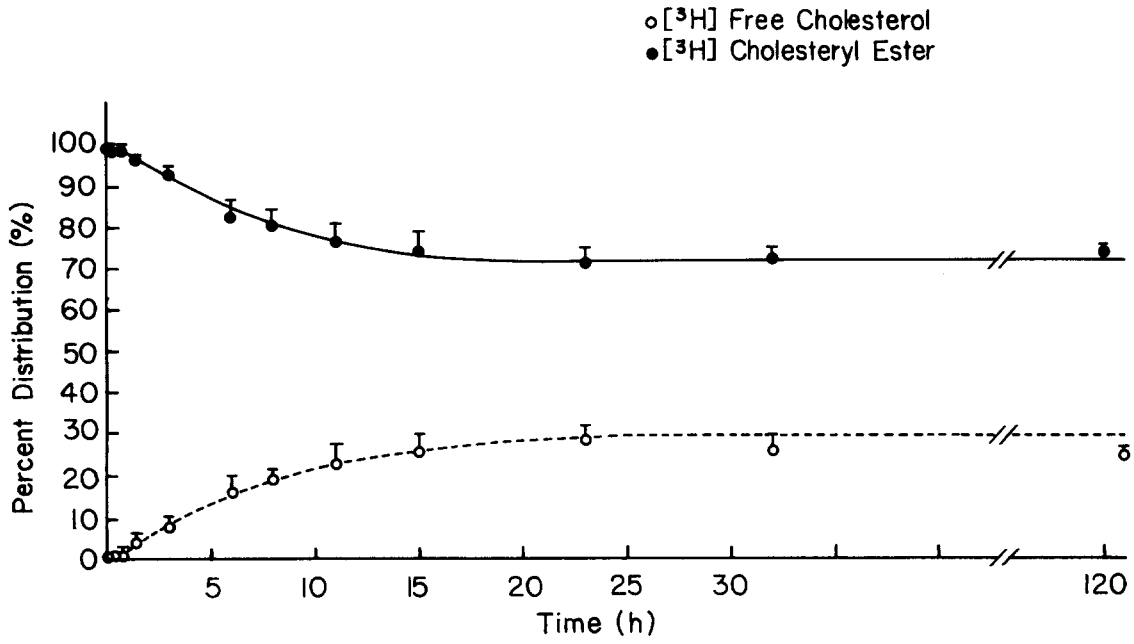

Fig. 6. Distribution of [^{3}H]cholesterol following reinjection of [^{3}H]cholesteryl [^{14}C]oleate-labeled monkey plasma lipoproteins. The free [^{3}H]cholesterol and [^{3}H]cholesteryl ester radioactivity from TLC was expressed as a percent of the total radiolabeled cholesterol. Each point represents mean ± SD, n = 2. Unesterified and esterified cholesterol mass was also measured and found to remain constant as 26 and 74% of total cholesterol, respectively.

showed that less than 3% of the cholesterol moiety of plasma cholesteryl ester was converted into free cholesterol during 8 hr of in vitro incubation. We have carried out an in vitro study in which we have demonstrated a relatively linear production of radioactive free cholesterol from [^{3}H]cholesteryl oleate over a 5-hr period of in vitro incubation (M. S. Thomas and L. L. Rudel, unpublished results, and M. S. Thomas, Ph.D. thesis, Bowman Gray School of Medicine of Wake Forest University, 1984). However, this reaction occurred at a rate some 10–15 times slower than the in vitro rate of cholesterol esterification by LCAT; thus it would not appear to be responsible for the major difference between the clearance rates of the cholesterol and fatty acid moieties of plasma cholesteryl esters. However, the extent to which these in vitro conditions mimic the in vivo situation is unknown and the possibility remains that some of the labeled free cholesterol appearing in vivo may arise via this route in plasma.

Specific uptake and hydrolysis of lipoprotein cholesteryl esters by the liver has been described after in vivo injection of chylomicrons in the rat by Goodman and Quarfordt (20) and after in vitro perfusion of cholesteryl ester-labeled plasma lipoproteins through the rat liver by Goodman and LeQuire (34). It is our assumption that the majority of the cholesteryl ester removed from plasma in our experiments was also removed by the liver and underwent a similar hydrolysis. However, the fact that some 60% of the ester cholesterol removed reappeared in plasma suggests that the cholesterol generated by hydrolysis was not efficiently equilibrated with liver cholesterol pools, but was selectively resecreted into the plasma compartment. This suggests that compartmentalization of cholesterol in the liver must occur. In this case it is appealing to hypothesize that the hydrolysis occurs in the plasma membrane so that the cholesterol moiety has maximum access to the plasma compartment. In this context, it is interesting to note that in the cell fractionation experiments of Goodman and LeQuire (34), the plasma membrane fraction was found to contain the greatest relative amount of radioactivity of any liver subcellular fraction.

Since the liver is believed to be the primary site for uptake and hydrolysis of plasma cholesteryl esters, we have calculated the extent to which the incoming plasma ester cholesterol could be diluted by hepatic cholesterol pools. A typical African green monkey weighing 4 kg has a liver weighing about 85 g. Cholesterol secretion by such a liver during perfusion is about 8 mg of cholesterol per hr (5). A liver this size contains about 400 mg of total cholesterol or about 250 mg of free cholesterol and 150 mg of cholesteryl ester (4). Total hepatic cholesterol output per day can be assumed to be the sum of that secreted into plasma with lipoproteins (8 mg/hr) and that secreted into bile (8 mg/hr) or about 16 mg/hr. In the steady state, hepatic output will be balanced by input and, assuming that hepatic synthesis is low in cholesterol-fed monkeys, input should approximate 16 mg/hr. In this example, only the cholesteryl ester is labeled and cholesteryl ester is 75% of

TABLE 2. Distribution of [^{3}H]cholesterol among plasma lipoprotein cholesteryl esters following reinjection of [^{3}H]cholesteryl [^{14}C]oleate-labeled lipoproteins

	% Distribution of ^{3}H-Labeled Sterol Radioactivity[a]				
Time	(20:4) + (18:3)[b]	(18:2)	(18:1)	(16:0)	(18:0)
hr					
1			99.5		
3		1.1	98.9		
6		4.1	92.9		1.0
10	1.5	8.9	87.3		1.0
15	2.3	11.3	70.4	11.5	4.3
24	2.4	19.6	59.1	12.4	4.7
48	4.0	24.5	55.8	11.6	3.8

[a] These values represent the average of plasma radioactivity from four different animals at each time point.

[b] Chol(20:4), cholesteryl arachidonate; chol(18:3), cholesteryl linolenate; chol(18:2), cholesteryl linoleate; chol(18:1), cholesteryl oleate; chol(16:0), cholesteryl palmitate; chol(18:0), cholesteryl stearate.

the total plasma cholesterol, so that only a total of 12 mg of ester cholesterol could be generated by uptake and hydrolysis of lipoprotein cholesteryl ester each hour. Since the liver free cholesterol pool contains about 250 mg, the uptake of 12 mg per hr of ester cholesterol from plasma would not be expected to result in the reappearance in plasma of 60% of this cholesterol, assuming equilibration with the available liver free cholesterol pools. However, this high percentage of reutilization could occur if one assumes compartmentalization among the liver cholesterol pools. It should be noted that these calculations were made using numbers derived from perfused livers, and therefore necessarily only estimate true in vivo rates. However, in these studies (4, 5) a high correlation between plasma cholesterol concentrations in the liver-donor animals and hepatic cholesterol production rates during perfusion were found suggesting that the in vitro rate during perfusion reflects the in vivo situation.

Evidence for cholesterol reutilization in humans has been proposed by several investigators who have compared the daily cholesterol turnover in humans, which is 10–12 mg/kg per day, to the plasma cholesteryl ester turnover which is about 25 to 30 mg/kg per day (35–38). Therefore, the production of cholesteryl ester in plasma is about 2–3 times greater than the amount of total cholesterol turned over in 1 day and this is believed to represent cholesterol reutilization. The data of the present study show that total plasma cholesterol turnover in African green monkeys is about 15 mg/kg per day which is comparable to the published value of 20 mg/kg per day (2) while cholesteryl oleate turnover was found to be over 2 times greater or 46 mg/kg per day (data derived from Table 1). Therefore, it seems reasonable to assume that efficient return to plasma of cholesterol taken out of plasma as cholesteryl ester is an important component of whole body cholesterol metabolism and worthy of additional study.

This research was supported in part by NIH grants HL-14164 and HL-24736 and by a grant from the North Carolina Heart Association and an R. J. Reynolds Industries, Inc. Special Fellowship. We wish to thank Lauren Clarkson for the expert technical assistance during the reinjection studies and Linda Odham for assistance in the preparation of this manuscript. Portions of this work have appeared in abstract form: Thomas, M. S., and L. L. Rudel. Differential fates of doubly labeled cholesteryl oleate in plasma. *Federation Proc.* 1984; **43:** 1543; and Thomas, M. S., and L. L. Rudel. Cholesterol esters do not remain intact in the circulation. *Arteriosclerosis.* 1984; **4:** 566a.

Manuscript received 31 October 1986 and in revised form 7 January 1987.

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