Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans

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Abstract **Our aim was to identify and quantify the major in vivo pathways of lipoprotein cholesteryl ester transport in humans. Normal (n = 7), bile fistula (n = 5), and familial hypercholesterolemia (FH; n = 1) subjects were studied. Each received isotopic free cholesterol in HDL, LDL, or particulate form, along with another isotope of free or esterified cholesterol or mevalonic acid. VLDL, intermediate density lipoprotein (IDL), LDL, HDL, blood cells, and bile were collected for up to 6 days for analysis of radioactivity and mass of free and esterified cholesterol. These raw data were subjected to compartmental analysis using the SAAM program. Results in all groups corroborated net transport of free cholesterol to the liver from HDL, shown previously in fistula subjects. New findings revealed that 70% of ester was produced from free cholesterol in HDL and 30% from free cholesterol in LDL, IDL, and VLDL. No evidence was found for tissue-produced ester in plasma. There was net transfer of cholesteryl ester to VLDL and IDL from HDL and considerable exchange between LDL and HDL. Irreversible ester output was from VLDL, IDL, and LDL, but very little was from HDL, suggesting that selective and holoparticle uptakes of HDL ester are minor pathways in humans. It follows that *1)* they contribute little to reverse transport, *2)* very high HDL would not result from defects thereof, and *3)* the clinical benefit of high HDL is likely explained by other mechanisms. Reverse transport in the subjects with bile fistula and FH was facilitated by ester output to the liver from VLDL plus IDL.**—Schwartz, C. C., J. M. VandenBroek, and P. S. Cooper. **Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans.** ***J. Lipid Res.*** **2004.** 45: **1594–1607.**

Supplementary key words cholesterol • high density lipoprotein • kinetics • low density lipoprotein • reverse transport

Approximately 70% of cholesterol molecules in normal human plasma are esterified, mostly to long-chain fatty acids (1, 2). Cholesteryl ester is insoluble in water and is a major component of the hydrophobic core of all plasma lipoprotein particles. Its relevance in human pathophysiology is highlighted by atherosclerosis, the sequel of low HDL or high intermediate density lipoprotein (IDL) and LDL cholesterol.

Cholesteryl ester is produced by LCAT from free cholesterol on the surface of plasma HDL and resides in the core of the HDL particle (1, 2). Ester in the core of plasma VLDL is believed to originate by transfer from HDL and from the hepatocyte, where lipids are assembled into VLDL (3). The ester may remain in the core while a VLDL particle is delipidated to IDL and then to LDL (3). In 1978, Pattnaik and colleagues (4) challenged the notion that ester molecules were core-locked within any human lipoprotein. In the presence of certain plasma proteins in vitro, esters readily transfer to VLDL from HDL and LDL and exchange between HDL and other lipoproteins (5). Cholesteryl ester transfer protein (CETP) was identified in 1989 (6). It can remodel the composition, size, and function of lipoproteins by exchanging HDL ester for VLDL triglyceride, for example (5, 7); it can also promote a futile cycle of bidirectional ester exchange (8). Lipid transfer inhibitor protein (LTIP) can modify ester transport between lipoproteins in vitro (9). Finally, irreversible cholesteryl ester output occurs by intracellular hydrolysis to free cholesterol. Cell entry is gained by endocytosis of an entire lipoprotein particle or by selective uptake of ester from a circulating particle (10–12).

Whether the above pathways of lipoprotein cholesteryl ester metabolism are inclusive, are regulated, or even occur in vivo in humans is largely unknown. Here, we report in vivo studies designed to identify and quantify all major pathways of lipoprotein cholesteryl ester transport, such as to the liver where output ultimately occurs. This was ac-

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Abbreviations: apoB, apolipoprotein B; CETP, cholesteryl ester transfer protein; C(m), compartment m in the model; C(n), compartment n in the model; FH, familial hypercholesterolemia; FSD, fractional SD (SD ÷ value); IDL, intermediate density lipoprotein; LTIP, lipid transfer inhibitor protein; L(m,n), the fraction of cholesterol in C(n) transferred to C(m) per minute; M(n), the mass of C(n) in micromoles; R(m,n), micromoles of cholesterol transported to C(m) from C(n) per minute; SR-BI, scavenger receptor class B type I.

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complished using isotopes and compartmental analysis. The approach was plausible because a comprehensive model has been developed for free cholesterol (13), the immediate precursor and final product of cholesteryl ester.

METHODS

Subjects

Seven normal subjects, five with bile fistula, and one with homozygous familial hypercholesterolemia (FH; LDL receptor negative) were studied after giving informed consent with approval from the Medical College of Virginia human research committee. All 13 subjects were ambulatory on an ad libitum diet for at least 10 days before admission to the clinical research center on the evening before isotope administration. Portions of the data from four normal subjects and four subjects with bile fistula (identified in **Table 1**) have been published. The published data include mass and radioactivity in all lipoprotein free cholesterol fractions plus HDL, LDL, and whole plasma cholesteryl ester. The new data presented here from these eight subjects include cholesteryl ester mass and radioactivity in IDL, VLDL, β- and α-lipoprotein, and additional HDL and LDL samples. Bile and blood cell data were published only for the four subjects with fistula.

Clinical information is summarized in Table 1 and **Table 2**. Triglyceride was less than 190 mg/dl in each subject. Hematocrit was 39–47% in all subjects, except it was 31% in ID and 34% in FH-A. All 13 subjects had normal blood glucose and normal renal and liver function except for mild (<2-fold) elevation of alkaline phosphatase in some subjects with bile fistula. The minimum to maximum body weight of each subject varied by less than 1.5% from the fifth day preceding the study to the day of completion.

Experiments

Subjects were administered the isotope by vein over 90 s at approximately 11 AM; a second isotope was administered immediately after the first. Subjects fasted except for water for 12–14 h before and 6–10 h after isotope administration. Blood samples (5–10 ml) were collected in EDTA (1 mg/ml) or heparin (143 United States Pharmacopeia units). Plasma was removed from cells after centrifugation of blood at 3,000 rpm and 5°C starting within 2 min of venepuncture. The cells were quickly washed three times with cold saline and extracted, as were all samples, in 20 volumes of chloroform-methanol (2:1, v/v). Occasional EDTA-

TABLE 1. Labeled preparations administered and miscellaneous clinical information

Subject	Labeled Preparation[a]	dpm × 10^{-6} Injected: Free	dpm × 10^{-6} Injected: Esterified[b]	Clinical Information[c]
SWI	**HDL [^{3}H]cholesterol**[d]	19.29	8.31 (30.1%)	Subject 4 in ref. (18); bile fistula open for 7 days; prior tubal ligation
	HDL [^{14}C]cholesterol	1.48	0.004 (0.3%)	
SWII	**LDL [^{3}H]cholesterol**	40.10	2.85 (6.6%)	
	LDL [^{14}C]cholesterol	1.07	0.004 (0.4%)	
IDI	HDL [^{3}H]cholesterol	38.73	0.48 (1.2%)	Subject 2 in ref. (18), I.D. in ref. (13); bile fistula open for 14 d
	LDL [^{14}C]cholesterol	1.02	0.001 (0.1%)	
IDII	HDL [^{14}C]cholesterol	0.72	0.034 (4.5%)	
	LDL [^{3}H]cholesterol	26.33	0.050 (0.2%)	
EBI	HDL [^{3}H]cholesterol	47.26	0.42 (0.9%)	Subject 1 in ref. (18), E.B. in ref. (13); bile fistula open for 4 d
	LDL [^{14}C]cholesterol	1.32	0.005 (0.4%)	
EBII	HDL [^{14}C]cholesterol	0.40	0.017 (4.1%)	
	LDL [^{3}H]cholesterol	57.48	0.085 (0.1%)	
EHI	DL[5-^{3}H]mevalonic acid	NA[e]	477.30[f]	Subject 6A in ref. (18), E.H.I in ref. (13); bile fistula open for 5 d
	Albumin [^{14}C]cholesterol	35.00	0.000 (0.0%)	
GM	DL[5-^{3}H]mevalonic acid	NA	248.10[f]	Bile fistula open for 5 d; no medication for 3 weeks
	Albumin [^{14}C]cholesterol	34.63	0.000 (0.0%)	
GAIII	**HDL [^{3}H]cholesterol**	10.56	4.98 (32.1%)	Subject 2 in ref. (16), 5A in ref. (18); cholecystectomy 3 years prior
	HDL [^{14}C]cholesterol	2.62	0.007 (0.3%)	
DB	HDL [^{3}H]cholesterol	33.16	0.16 (0.5%)	Subject 3 in ref. (18); cholecystectomy 3 years prior
	LDL [^{14}C]cholesterol	1.81	0.003 (0.2%)	
GAII	DL[5-^{3}H]mevalonic acid	NA	318.13[f]	Subject 5B in ref. (18)
EHII	DL[5-^{3}H]mevalonic acid	NA	260.85[f]	Subject 6B in ref. (18); bile fistula closed 3 months prior
	Albumin [^{14}C]cholesterol	30.93	0.000 (0.0%)	
MV	DL[2-^{14}C]mevalonic acid	NA	77.83[f]	
	HDL [^{3}H]cholesterol	62.31	0.65 (1.0%)	
WR	DL[2-^{14}C]mevalonic acid	NA	101.00[f]	
	HDL [^{3}H]cholesterol	31.45	0.20 (0.6%)	
HR	DL[2-^{14}C]mevalonic acid	NA	76.40[f]	Cholecystectomy and tubal ligation 10 years prior
	HDL [^{3}H]cholesterol	19.78	0.22 (1.1%)	
FH-A	DL[2-^{14}C]mevalonic acid	NA	94.54[f]	Coronary and cerebral artery disease; selective plasma filtration 17 d prior[g]
	LDL [^{3}H]cholesterol	59.80	0.11 (0.2%)	

[a] HDL preparations isolated at d = 1.065–1.21 g/ml, except in GAIII and DB, for whom the HDL preparation is from the d = 1.21 g/ml top of the heparin-$MnCl_2$ supernate (18). LDL isolated at d = 1.019–1.063 g/ml. Albumin [^{14}C]cholesterol was in particulate form (13).

[b] The percentages of injected isotopic cholesterol esterified are in parentheses.

[c] SW, ID, and EB had a T-tube inserted during cholecystectomy and bile duct exploration for gallstones. A T-tube was inserted during bile duct surgery for benign mass and trauma in EH and GM at least 4.5 weeks before the study.

[d] Preparations that intentionally contain isotopic ester are shown in boldface.

[e] NA, not applicable.

[f] Free and esterified do not apply. The number represents dpm × 10^{-6} mevalonic acid injected.

[g] Selective filtration was done every 2–3 weeks for 6 months before the study. FH-A's plasma LDL cholesterol concentration increased by ~1% per day, and HDL did not change at 15–20 days after filtration.

TABLE 2. Information on subjects and cholesterol concentrations (μmol × 0.3866 = mg)

Subject	Sex	Age	Weight	Height	Duration[a]	Free Cholesterol[b] BC[c]	HDL	VLDL	IDL	LDL	Cholesteryl Ester[b] HDL	VLDL	IDL	LDL
Bile fistula		*years*	*kg*	*cm*	*min*					*μmol/100 ml*				
SWI and II	Female	33	100.5	158	2,002/1,605	185	22.5	40.9	13.1	54.0	77.5	45.5	17.9	133.7
IDI and II	Female	68	61.8	167	5,525/4,280	176	22.2	29.2	12.9	47.1	75.2	26.9	18.2	112.8
EBI and II	Male	59	74.6	180	5,558/4,315	218	25.8	18.8	9.5	55.1	87.7	22.1	17.1	133.1
EHI	Male	49	86.8	186	8,775	241	15.7	14.8	50.5[d]		45.3	17.1	117.9[d]	
GM	Male	20	68.3	170	3,025	194	23.2	8.0	26.2[d]		70.0	8.8	52.1[d]	
Normal														
GAIII	Male	86	57.7	165	5,645	213	27.7	10.7	9.0	86.4	79.4	11.9	11.2	210.0
DB	Male	60	77.7	174	600	230	35.2	11.5	7.5	75.2	105.9	13.6	10.5	206.2
GAII	Male	84	56.4	167	5,694	211	28.0	9.5	5.6	97.9	92.4	8.8	6.9	245.0
EHII	Male	49	86.8	186	5,485	226	21.0	9.4	72.0[d]		55.0	11.1	156.6[d]	
MV	Male	25	70.2	175	5,660	231	24.3	11.2	74.7[d]		91.2	13.0	211.1[d]	
WR	Male	29	69.6	173	1,804	196	26.7	1.7	65.9[d]		108.6	2.2	193.0[d]	
HR	Female	44	72.3	163	1,380	179	24.0	5.0	77.6[d]		78.4	3.9	225.2[d]	
FH														
FH-A	Female	48	61.8	160	1,515	142	14.5	5.2	5.5	311.0	73.1	10.7	14.8	847.1

FH, familial hypercholesterolemia; IDL, intermediate density lipoprotein.

[a] Duration of experiment starting with administration of the first isotope. In SW, ID, and EB, the time at which a second pair of labeled preparations was administered is shown after the slash.

[b] n = 16–28 for each cholesterol concentration in each subject except DB, for whom n = 11 for each cholesterol concentration.

[c] BC, blood cell free cholesterol concentration as μmol/100 ml plasma, calculated from hematocrit and μmol cholesterol/ml packed cells.

[d] IDL and LDL not separated; they were isolated together as d = 1.006–1.063 g/ml.

plasma samples were extracted directly. All other EDTA-plasma samples started ultracentrifugation at 5°C within 8 h of venepuncture to isolate lipoproteins. To retard ex vivo cholesteryl ester production and transfer during the processing of EDTA-plasma, 5,5′-dithiobis-(2-nitrobenzoic) acid was added to 0.02 M immediately after removal of cells; all samples, infranates, reagents, etc., were maintained at 4–5°C. Heparinized plasma was treated with heparin-$MnCl_2$ immediately after removal from cells to precipitate β-lipoprotein (14), thereby separating β- from α-lipoprotein within 10–12 min of venepuncture.

In six of the seven normal subjects and in FH-A, an enteral feeding tube was passed by mouth and the opening positioned in the third part of the duodenum with fluoroscopic guidance 3–4 h before isotope administration. The opening was used to aspirate 4 ml of bile in 2–3 min with a syringe at 15–60 min intervals for extraction. In subjects with a gallbladder, cholecystokinin octapeptide (Kinevac; Squibb Diagnostics, Princeton, NJ) was infused by vein at 1.0 μg/h while the enteral tube was in place to contract the gallbladder. Approximately 8 h after isotope administration, the infusion was stopped, the tube removed, and the subject's usual diet resumed. In GAIII, 4 ml of duodenal bile was aspirated on the three following mornings by insertion of the tube before breakfast. EHII had a permanent T-tube that was unclamped at intervals to obtain 4 ml of bile. Bile was collected continuously in the subjects with fistula (13).

Isotopes and labeled lipoprotein preparations

Isotopes were obtained from NEN Research Products and stored at −15°C in ethanol. Radiolabeled cholesterol was used only if >94% pure as determined by all three methods: silicic acid column chromatography (15), thin-layer chromatography (16), and precipitation as the digitonide (17). Radiolabeled mevalonic acid was administered as described previously (16). Three subjects were administered free [4-^{14}C]cholesterol with albumin in particulate form (13). Each labeled lipoprotein was autologous, prepared from fasting plasma obtained 2–4 days before administration (13, 16, 18).

Analysis of labeled lipoproteins revealed a protein, phospholipid, free cholesterol, and cholesteryl ester composition typical of the native lipoprotein. The distribution of isotope between free and esterified cholesterol in each preparation is shown in Table 1. When isotopic cholesterol was transferred to plasma from filter paper at 4–5°C, the fraction esterified in the final preparation was usually <1.2%; exceptions were ~4.3% of ^{14}C esterified in HDL of IDII and EBII. When transferred to plasma at 37°C to intentionally label esters (GAIII and SW), ~31% of the isotope in HDL was esterified but only 6.6% in LDL was esterified. Agarose gel electrophoresis of each preparation revealed a single visible band of LDL or HDL. The gels were sliced and radioactivity determined; in LDL preparations, 90–95% was in LDL and 3–5% was in slices corresponding to HDL; in HDL preparations, 82–90% was in HDL and 8–12% corresponded to LDL. Most preparations were ultracentrifuged at densities 1.019, 1.063, and 1.21 g/ml; the radioactivity was distributed as on the gels.

Analytic procedures

Chloroform and methanol were separated by the addition of 1/5 volume of water. The chloroform phase was subjected to silicic acid column chromatography to quantitatively isolate fractions of free and esterified cholesterol (15). Determination of free cholesterol mass and radioactivity was previously described, as was that of cholesteryl ester (13). ^{3}H and ^{14}C radioactivity were determined by liquid scintillation counting for 20 min or longer so that the error was ≤3%. Corrections were made for the loss of ^{3}H and ^{14}C during the conversion of isotopic mevalonate to cholesterol (19).

Multicompartmental analysis

Each subject was in steady state as supported by constant weight and cholesterol level. The final model was developed from the starting model using SAAM/CONSAM software (20). The topology of the model was constrained such that one set of rate constants simulate both ^{3}H and ^{14}C specific activity (dpm/μmol) observations in all intravascular cholesterol compartments and bile after administration of ^{3}H and ^{14}C preparations to different compartments. This could be accomplished for each subject using ^{3}H and ^{14}C models running simultaneously, with

steady-state input and rate constants of the ^{3}H model equal to those of the ^{14}C model.

The starting model shown in **Fig. 1** was derived from the free cholesterol model (13) plus the following. Intravascular cholesteryl ester compartments [C(n), compartment n in the model] were added for HDL [C(9)], LDL [C(7)], VLDL [C(8)], and IDL [C(10)]. Pathways to C(9) from C(4) and C(7) were previously defined (18). Pathways to C(8) and C(7) from C(9) were incorporated based on the in vivo study of Monroe, Vlahcevic, and Swell (21) and other evidence (5–9). Some ester molecules probably remain in the particle as C(8) matures to C(10) and then to C(7), and pathways were added accordingly (3). Cholesteryl ester output could be to liver [C(12)] or to extrahepatic pools such as C(6). Ester output could be from C(8), C(10), C(7), or C(9); there are data from humans and animals supporting all possibilities. Therefore, output to both C(12) and C(6) from all four lipoproteins was incorporated.

The total amount of isotopic free and esterified cholesterol administered to each subject is shown in Table 1. Most was allocated to the appropriate compartment, but a small amount of isotope was not in the intended lipoprotein, as found by analysis of agarose gels (see above); this small amount was allocated to the proper compartment. The technique for instantaneous distribution of [^{14}C]cholesterol administered in albumin, part of which was particulate, between C(4) and the reticuloendothelium [C(25) in Fig. 1], has been described (13). Efflux of [^{14}C]cholesterol to C(4) from C(25) was incorporated based on previous studies (3, 13).

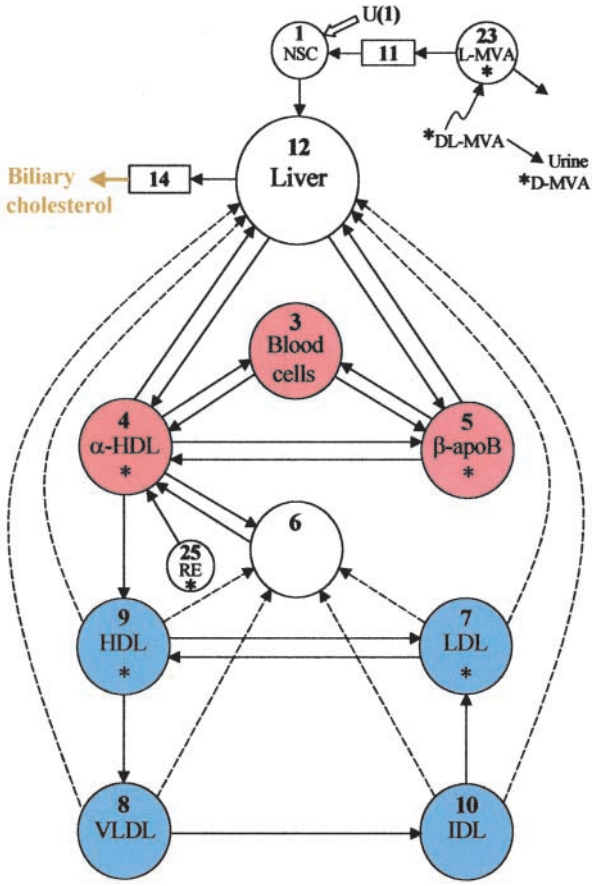

Fig. 1. The starting cholesterol model. Circles represent standard compartments and rectangles represent delay compartments, each identified within by a number; those with color were sampled. Red circles indicate a free cholesterol compartment in blood, blue circles indicate a cholesteryl ester compartment in blood, and the gold arrow indicates duodenal bile, also sampled. Arrows represent transport pathways; arrows for cholesteryl ester output pathways are dashed for contrast. U(1) and the block arrow represent cholesterol synthesis. α-HDL, the heparin-$MnCl_2$ supernatant; β-apoB (β-apolipoprotein B), the heparin-$MnCl_2$ precipitate; MVA, mevalonic acid; NSC, newly synthesized cholesterol; RE, reticuloendothelium. * A compartment in which isotope was administered.

In Fig. 1, U(1) is hepatic cholesterol synthesis and for simplicity herein represents total body cholesterol steady-state input. In FH-A and normal subjects, U(1) was fixed at 2.1 μmol/min/70 kg (22). In each bile fistula subject, U(1) was fixed at the subject's bile acid plus biliary cholesterol secretion rate (13). Ester kinetics were insensitive to a wide range of U(1) values. U(6) (extrahepatic synthesis or absorption) may occur to a major degree but was unidentifiable with the present design, whereas U(1) of at least 30% of total body input is essential to fit bile radioactivity after isotopic mevalonic acid administration (13).

The specific activity of biliary cholesterol was used to represent that of total body output (Fig. 1). This simplification was feasible because all biliary cholesterol and at least 93% of newly synthesized bile acids come directly from C(12) in fistula subjects (13) and in normal subjects (our unpublished observations). C(14) was named C(10) previously and represents a biliary transit delay (13).

The specific activity of cholesteryl ester in α-lipoprotein followed the same pattern as that in HDL; therefore, both were used to represent C(9). Within each ultracentrifuged sample, the specific activities of free cholesterol in VLDL, IDL, LDL, and HDL were essentially identical because of ex vivo exchange (13, 16). A linear combination of C(4) and C(5) [see component(45) in Fig. 4 of ref. (13)] was therefore used to fit the free cholesterol activities in VLDL, IDL, LDL, HDL, and whole plasma. Other linear combinations were used: a combination of C(10) and C(7) was used for cholesteryl ester specific activity in lipoproteins of d = 1.006–1.063 g/ml (IDL not separated from LDL); a combination of C(8), C(10), and C(7) was used for ester activity in β-lipoprotein; a combination of C(9), C(8), C(10), and C(7) was used for ester activity in whole plasma.

The estimate for the mass of a compartment [M(n)] in plasma was calculated from the mean plasma concentration (Table 2) and the plasma volume as 4.5% of body weight. M(5) was estimated from the sum of free cholesterol in VLDL, IDL, and LDL. Because the SEM of the cholesterol concentrations never exceeded 6% and was usually 2–3%, the estimate for M(3), M(4), M(5), M(7), M(8), M(9), and M(10) was weighted heavily during the iterative process; each final solution was 97–103% of its estimate. In each subject, the solutions were never all above or all below the estimates, supporting the 4.5% assumption.

Studies I and II of subjects SW, ID, and EB were separated by only 1–3 days and were in the same steady state. All ^{3}H and ^{14}C data from studies I and II therefore were fit in continuity by introducing the second pair of labeled preparations at the appropriate time after the first pair, yielding one set of rate constants.

RESULTS

Blood cholesterol concentrations

Only major differences merit comment because the groups were small and not homogeneous. Esterified and free cholesterol concentrations were higher in VLDL and lower in LDL of bile fistula subjects than of normal subjects (Table 2). LDL esterified and free cholesterol con-

centrations were higher in FH-A. The lipoprotein isolation technique did not influence the free or ester concentration. The concentration of cholesteryl ester in whole plasma (data not shown) was within 7% of the sum of esters in HDL, VLDL, IDL, and LDL. The β-lipoprotein ester concentration (data not shown) was within 7% of the sum of esters in VLDL, IDL, and LDL. The α-lipoprotein ester concentration (data not shown) was within 10% of HDL.

Specific activity time courses after administration of isotopic free cholesterol and mevalonic acid

The time courses for free cholesterol specific activity in α- and β-lipoprotein, whole plasma, blood cells, and bile followed the same patterns in normal subjects (**Figs. 2**, **3**) and FH-A (**Fig. 4**) as in fistula subjects described previously (13, 16) and shown in **Fig. 5**. These include *1*) the difference between α-lipoprotein activity and β-lipoprotein activity during the initial 150 min (13, 16) and *2*) the hump-like feature that interrupts the decline in plasma activity between 150 and 500 min seen only after the administration of particulate [^{14}C]cholesterol [lower panel of Fig. 7 in ref. (18)].

Several patterns were observed in the cholesteryl ester time courses (Figs. 2, 3B, 4, 5). *1*) There were no delays. Radioactivity increased in the cholesteryl esters of all four lipoproteins immediately after it appeared in free cholesterol. *2*) Initially, the specific activity of ester in HDL increased most rapidly. It changed to resemble a plateau ~150 min after isotopic free cholesterol was administered in HDL, 200 min after it was administered in LDL, 500 min after isotopic mevalonic acid, and 900 min after particulate [^{14}C]cholesterol. *3*) The specific activity of ester in VLDL reached that of HDL over a wide range of time (20–700 min) after isotope administration. IDL activity consistently reached HDL ~200 min after VLDL reached HDL. LDL reached HDL ~750 min after IDL. *4*) After reaching that of HDL, the ester specific activities in VLDL and IDL remained about the same as HDL at subsequent times, and LDL was just 3–5% higher. *5*) Cholesteryl ester specific activities of all four lipoproteins were nearly equal for a span of 0–500 min in the fistula group and for 500–1,500 min normally before intersecting the plasma free cholesterol specific activity; the LDL ester peak activity occurred near this intersection. These patterns suggest that a large portion of plasma ester is synthesized in HDL from HDL free cholesterol and that there is considerable ester exchange with or transfer to other lipoproteins. The only glaring difference between FH-A (Fig. 4) and other subjects was the failure of esterified and free cholesterol spe-

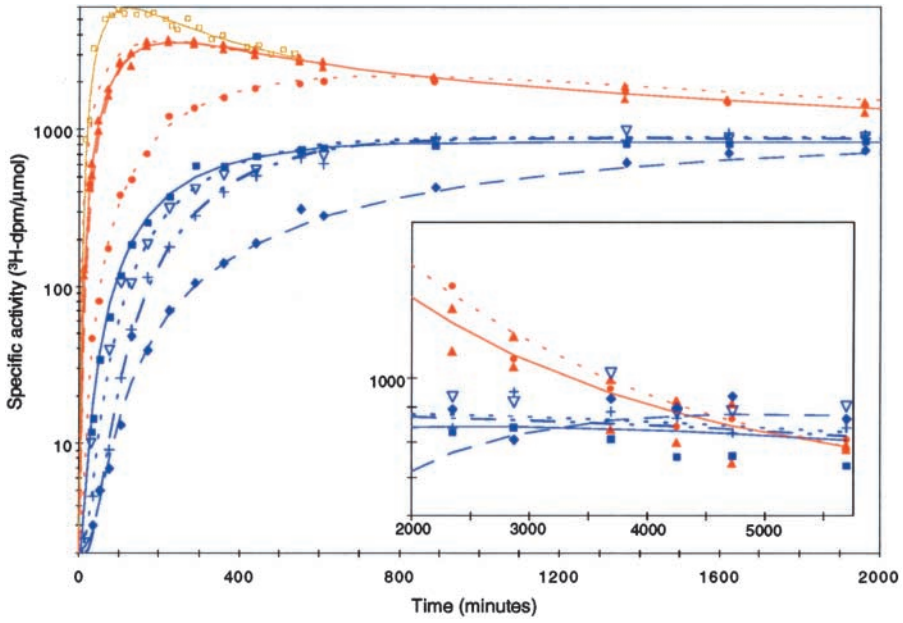

Fig. 2. Subject GAII. Specific activity-time courses after administration of DL[5-^{3}H]MVA. Blue symbols and lines represent cholesteryl ester observations and computer simulations, respectively. HDL, closed squares and solid line; VLDL, open down triangles and dotted line; intermediate density lipoprotein (IDL), plus and dot/dash line; LDL, closed diamonds and dashed line. Red symbols and lines represent blood free cholesterol observations and simulations, respectively. α-HDL, open circles and dotted line; β-apoB, asterisks and dashed line; whole plasma [a linear combination of C(4) and C(5)], closed triangles and solid line; blood cells, closed circles and dotted line. Gold symbols (open squares) and line represent bile free cholesterol observations and simulation, respectively. The model shown in Fig. 6 was used to obtain the computer-simulated specific activity-time courses with the SAAM program. For clarity, only two of the four observations representing whole plasma free cholesterol specific activity (closed triangles) are shown from each plasma sample that underwent ultracentrifugation to isolate VLDL, IDL, LDL, and HDL. The observations and simulation for whole plasma cholesteryl ester are not shown. In subject GAII, α-HDL and β-apoB were not isolated.

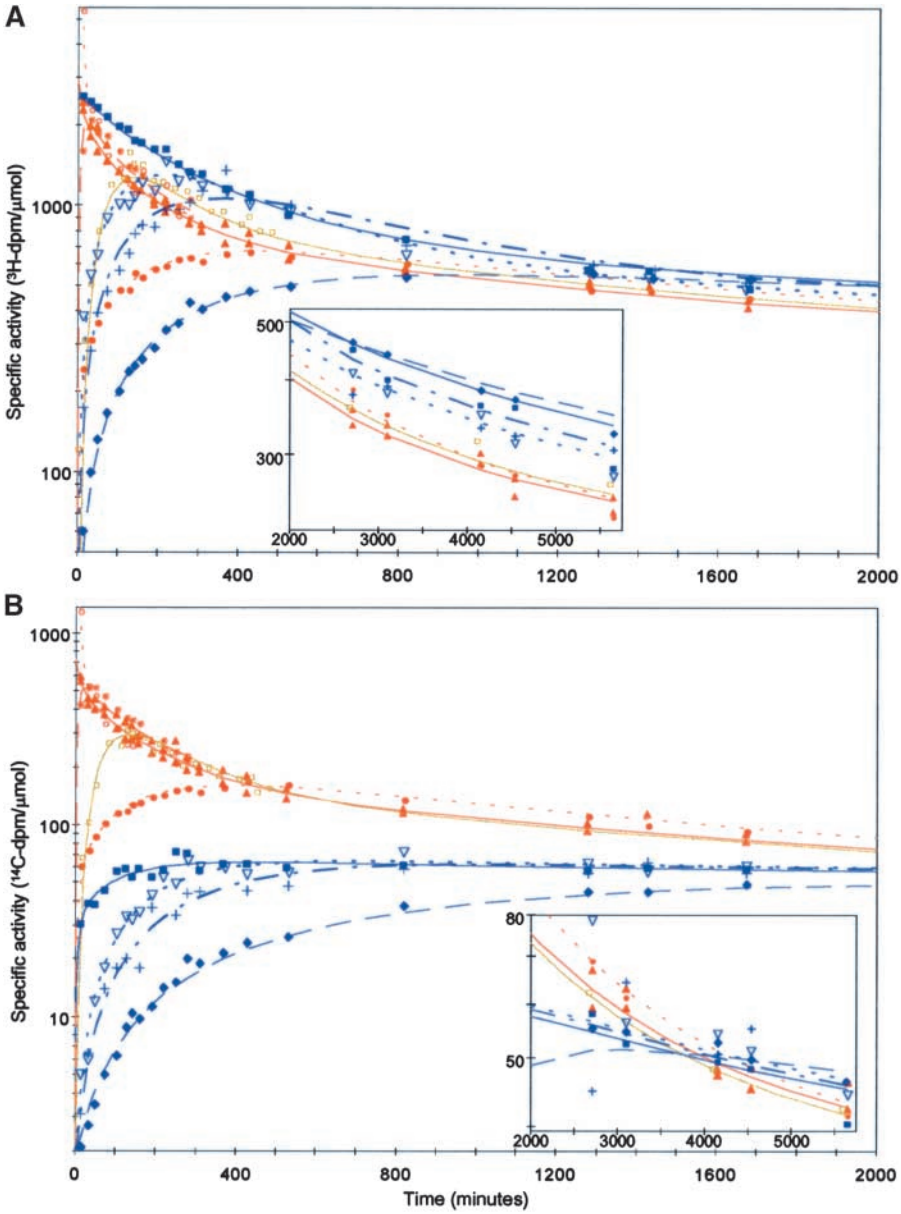

Fig. 3. Subject GAIII. Specific activity-time courses after simultaneous administration of [^{3}H]cholesteryl ester with free [^{3}H]cholesterol in HDL (A) and free [^{14}C]cholesterol in HDL (B). Colors, symbols, lines, and comments are as in the legend to Fig. 2. The ^{3}H and ^{14}C specific activity observations were simultaneously fit with one set of rate constants. Observations and simulation of β-lipoprotein cholesteryl ester are not shown.

cific activities to approach each other by 1,500 min. Thus, ester exchange is probably not diminished in FH, but the fractional turnover of the entire ester pool is low.

Specific activity time courses after administration of [^{3}H]cholesteryl ester with free [^{3}H]cholesterol in HDL and LDL

After administration in HDL (GAIII and SWI), HDL [^{3}H]ester specific activity decreased continuously (Fig. 3A). HDL free [^{3}H]cholesterol activity decreased faster, falling below HDL ester in ~30 min. The peak specific activities of VLDL, then IDL, and then LDL ester came close to intersecting the HDL ester activity and were higher than the plasma free cholesterol activity.

After administration in LDL (SWII), LDL [^{3}H]ester specific activity changed little from time zero (18). HDL activity, then VLDL and IDL activities, increased rapidly and equaled LDL activity by 300 min. The specific activity of plasma free [^{3}H]cholesterol was higher for the entire 400 min (18), showing the ineffectiveness of the technique for labeling LDL esters. However, all studies show that VLDL, IDL, and LDL ester activities follow HDL in a consistent pattern.

Evolution of the final model

L(m,n) identifies a pathway to C(m) from C(n) in the model and is also called a rate constant. Its value is the fraction of cholesterol in C(n) transported to C(m) per

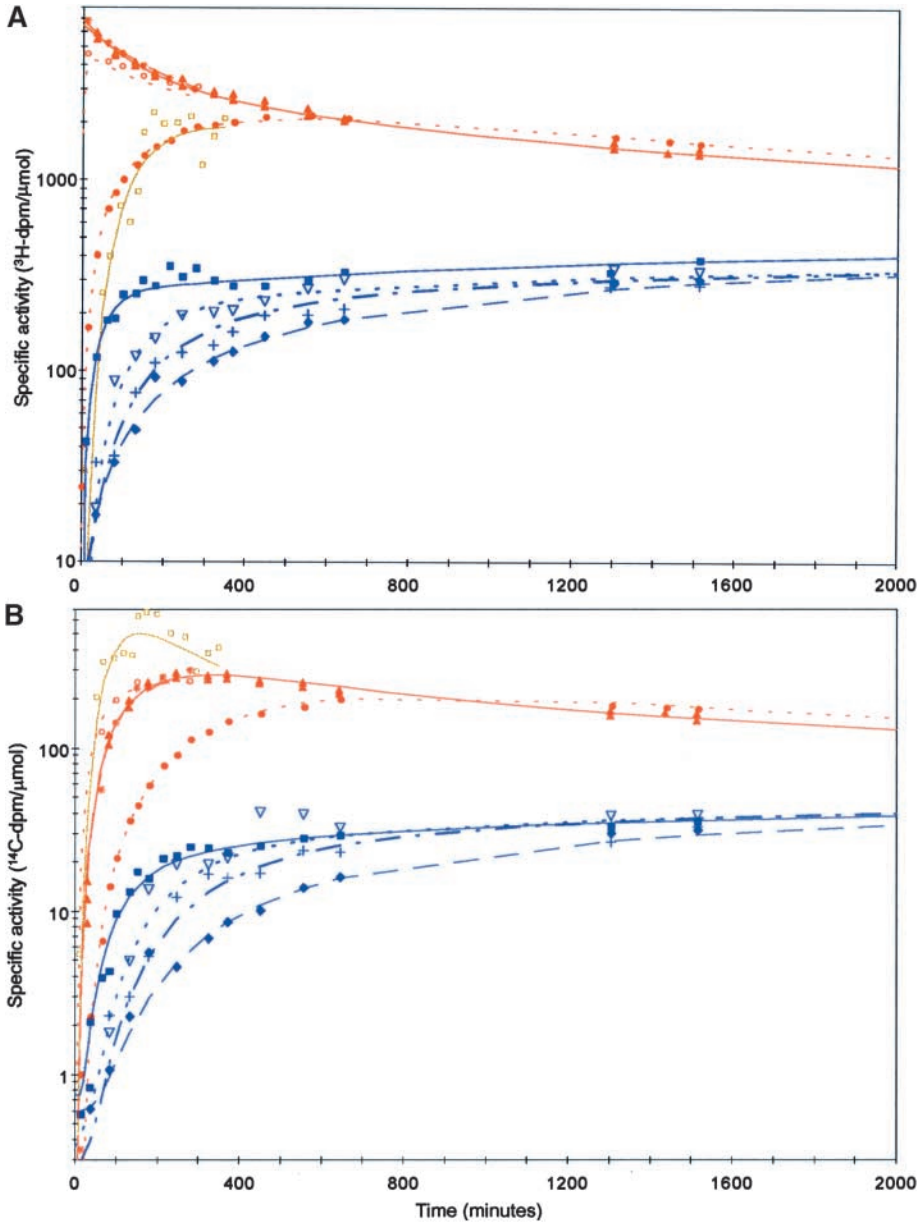

Fig. 4. Subject FH-A. Specific activity-time courses after simultaneous administration of free [^{3}H]cholesterol in LDL (A) and DL[2-^{14}C]MVA (B). Colors, symbols, lines, and comments are as in the legends to Figs. 2 and 3.

minute. The value for R(m,n) indicates the micromoles of cholesterol transported to C(m) from C(n) per minute.

The starting model was unable to simulate the rapid appearance of isotope observed in LDL, IDL, and VLDL cholesteryl ester during the initial 60 min after administration of isotopic mevalonic acid and free cholesterol. The data for subjects ID, EB, and DB, simultaneously administered free [^{3}H]cholesterol in HDL and free [^{14}C]cholesterol in LDL and then vice versa 1–3 days later, were most definitive for LDL ester. They showed that a pathway to LDL ester from β-lipoprotein free cholesterol [L(7,5)] resolved the discrepancy in LDL of all subjects. L(7,5) was added with confidence in part because the in vivo specific activity of LDL free cholesterol was observed closely by rapid isolation of β-lipoproteins that are LDL enriched. Pathways L(8,5) and L(10,5) resolved the discrepancy in VLDL and IDL esters. However, confidence in L(8,5) and L(10,5) was diminished without in vivo observations of VLDL and IDL free cholesterol activity; β-lipoprotein activity is probably a close approximation in most studies, as shown previously for VLDL (13, 16). Other support for the esterification of VLDL was found in unpublished data from three subjects administered free [^{3}H]cholesterol (or [^{14}C]cholesterol) in VLDL with free [^{14}C]cholesterol (or [^{3}H]cholesterol) in LDL; lipoproteins were then isolated only by precipitation. The fraction of ^{3}H dose was identical to the fraction of ^{14}C dose in β-lipoprotein esters in each sample, including those from the initial 60 min, indicating that esterification of VLDL is similar to that of LDL.

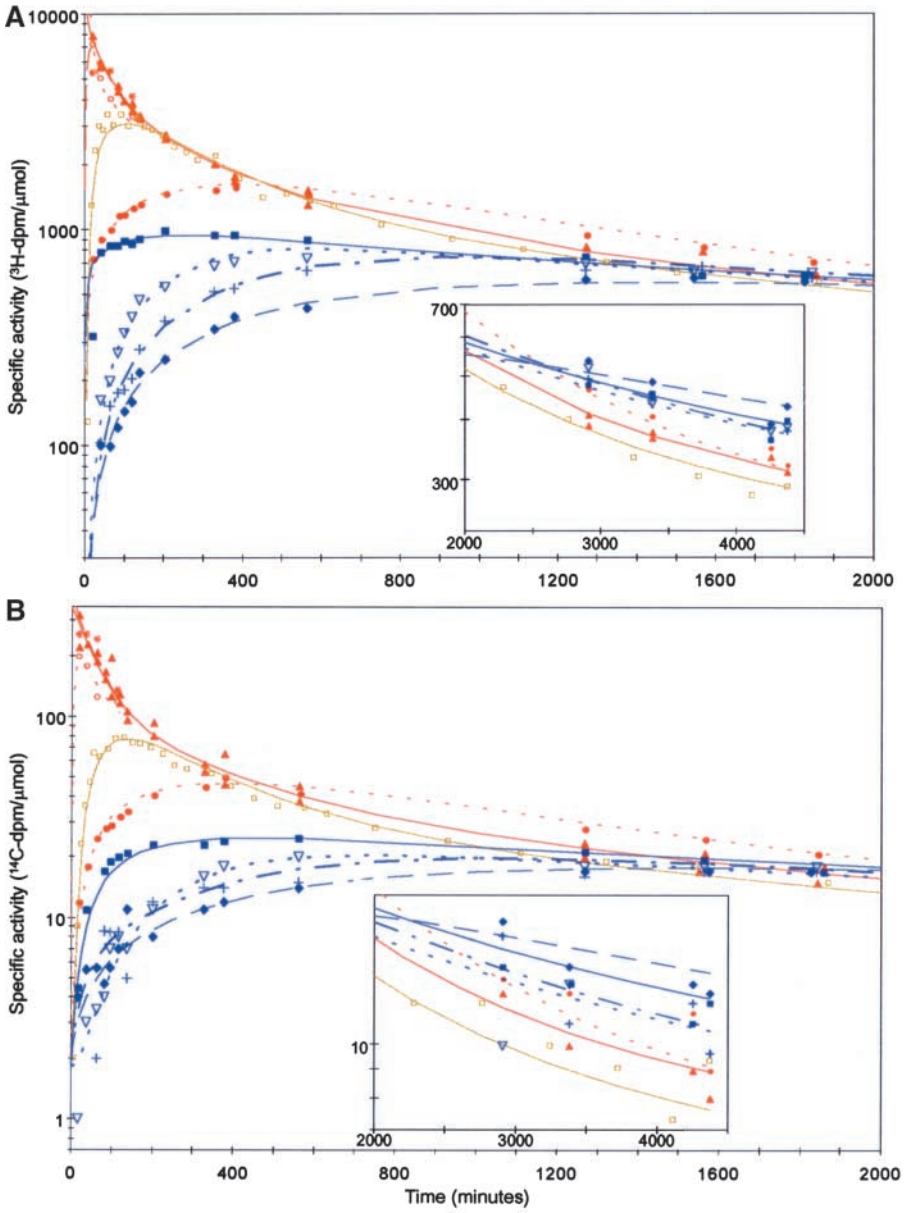

Fig. 5. Subject IDI. Specific activity-time courses after simultaneous administration of free [^{3}H]cholesterol in HDL (A) and free [^{14}C]cholesterol in LDL (B). Colors, symbols, lines, and comments are as in the legends to Figs. 2 and 3.

This modified starting model was unable to simulate M(8) and the observed VLDL ester specific activity as it rapidly increased between 75 and 300 min. The discrepancies were most apparent in subjects GAIII and SW, who had the more rigorous studies for identifying ester transfers by virtue of the [^{3}H]cholesteryl ester administered. Addition of a pathway to VLDL from LDL [L(8,7)] made the discrepancies worse. Adding L(9,8), as shown in **Fig. 6**, resolved both discrepancies and reduced the sum of squares in C(9) by ~2%.

This modified model was unable to simulate the rapid increase observed in IDL ester specific activity between 60 and 200 min, most notably in subjects GAIII and SW. Addition of a pathway to IDL from HDL [L(10,9)] resolved this discrepancy.

Simulations with and without the above additions gave a more rapid increase than were observed in all lipoprotein ester specific activities starting ~700 min after the administration of isotope. The simulated peak activities also occurred too early. An extravascular pool that exchanges with LDL ester, called C(17) in Fig. 6, resolved these discrepancies. An exchange pool with HDL, VLDL, or IDL ester did not help. M(17) was well defined [fractional SD (FSD) < 50%; FSD = SD ÷ value] in six subjects at ~60% of M(7) but was poorly defined in the other subjects. Therefore, M(17) was fixed at 60% of M(7) in all 13 subjects.

The irreversible output pathways (dashed arrows in Fig. 1) were examined at every step of model evolution. Path-

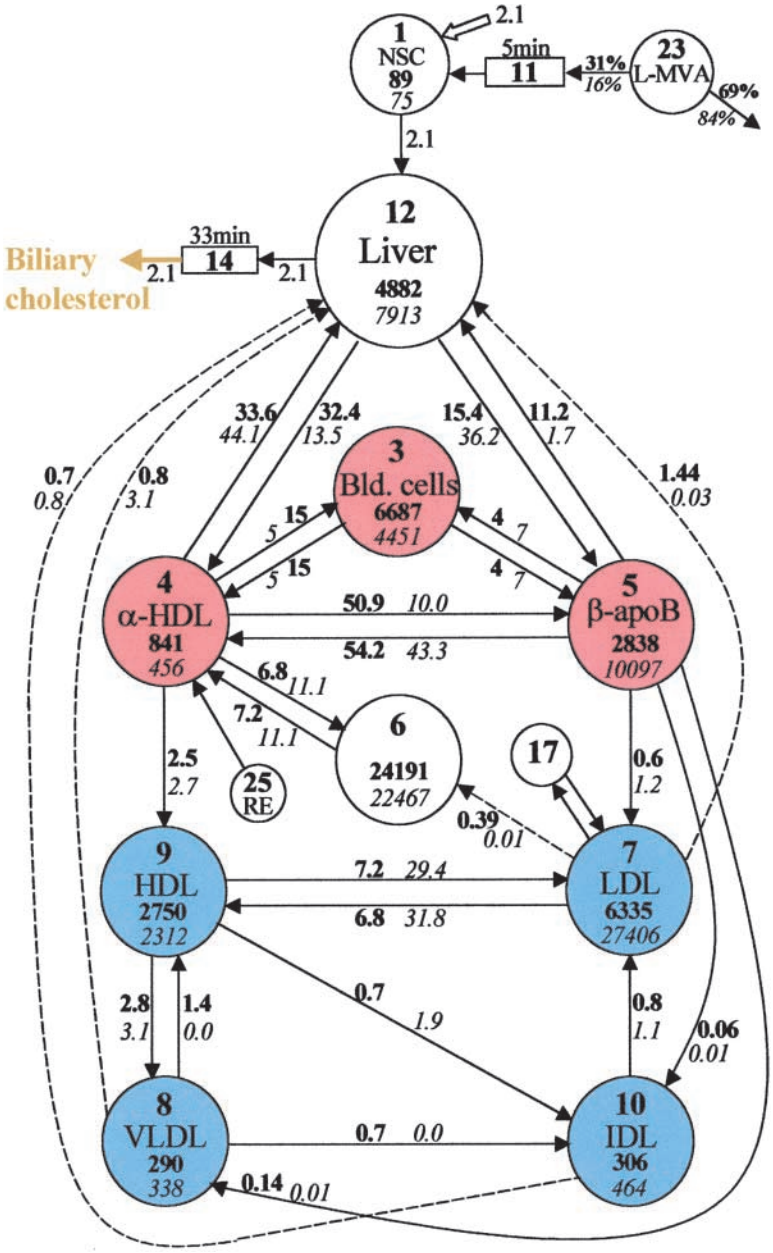

Fig. 6. The final cholesterol model. See Fig. 1 legend. Numbers adjacent to arrows and numbers in the lower half of the standard compartments are the solutions for transport (μmol/min/70 kg) and compartment size (μmol/70 kg), respectively; numbers in boldface are means for normal subjects; numbers in italic are for FH-A, the subject with familial hypercholesterolemia. The delay times shown above the rectangles varied little among subjects. The value of 2.1 μmol/min/70 kg for U(1), R(12,1), R(14,12), and R(0,14) was the same for FH-A and normal subjects. C(17) represents extravascular LDL cholesteryl ester. M(17) (data not shown) was 60% of M(7) in each subject; R(7,17) and R(17,7) are given in Table 4. The fraction of isotopic L-MVA converted to cholesterol is L(11,23). C(23) and C(25) contain only isotopic tracers (no mass) of L-MVA and cholesterol, respectively. Bld. cells, blood cells.

ways L(12,9), L(6,9), L(6,8), and L(6,10) were always undefined (FSD > 80%) and decreased toward zero during the iterative process. Therefore, they are absent in the final model (Fig. 6). With this model, no discrepancies occurred between the simulated and observed specific activities or pool sizes from subject to subject.

Final model parameter solutions

In the three subjects administered free [^{14}C]cholesterol in albumin (partly particulate), 76.4% (range, 73–82%) of total ^{14}C immediately entered the reticuloendothelium [C(25) in Fig. 6] and 23.6% entered C(4). Subsequent efflux of ^{14}C from the reticuloendothelium was only to plasma HDL as free cholesterol [L(4,25)], accounting for the hump-like feature in its specific activity. L(4,25) was very well defined (FSD < 6%) at 0.0020 min^{-1} (range, 0.0017–0.0024).

Results for free cholesterol transport in FH and normal subjects are shown in Fig. 6. Free cholesterol rate constants and transport in bile fistula and normal subjects were similar to those published previously for fistula subjects (13). The rate constants and transports that involve cholesteryl ester are shown in **Tables 3**, **4**. Differences between groups are not scrutinized closely because groups were small and not homogeneous and IDL was not separated from LDL in six subjects.

Production of cholesteryl ester from free cholesterol in each lipoprotein (Table 3), including IDL when isolated, was very well defined (FSD < 20%) with few exceptions. The rate constant for HDL ester production [L(9,4)] was 5- to 10-fold higher than that for the sum of VLDL, IDL, and LDL ester production [L(β,5)]. Presumably, L(9,4) and L(β,5) represent the α- and β-activities of LCAT, respectively. On an absolute basis (Table 4), ~70% of plasma

TABLE 3. Rate constants derived from compartmental analysis with the final model

Subject	L(9,4)	L(7,5)	L(10,5)	L(8,5)	L(β,5)[a]	L(7,9)	L(9,7)	L(8,9)	L(9,8)	L(10,9)	L(10,8)	L(7,10)	L(17,7)[b]	L(12,8)	L(12,10)	L(12,7)	L(6,7)[c]
SW	582 ± 19	76 ± 18	22 ± 23	22 ± 52	119	506 ± 11	279 ± 15	528 ± 9	866 ± 18	142 ± 10	70 ± 185	120 ± 295	280 ± 45	<1 ± 600	799 ± 29	52 ± 36	31
ID	388 ± 7	20 ± 12	6 ± 13	<1 ± 350	26	137 ± 17	107 ± 15	219 ± 8	224 ± 39	21 ± 52	95 ± 67	54 ± 120	42 ± 42	274 ± 12	200[d]	1 ± 395	<1
EB	345 ± 5	38 ± 8	5 ± 10	2 ± 15	46	189 ± 21	161 ± 18	249 ± 9	500 ± 30	42 ± 33	61 ± 190	116 ± 87	22 ± 49	425 ± 5	200[d]	<1 ± 600	<1
EHI	378 ± 8	35 ± 26	7[e]	<1 ± 600	42	364 ± 51	253 ± 12	444 ± 9	500 ± 108	73[f]	434 ± 115	420 ± 96	250 ± 44	245 ± 59	200[d]	9 ± 200	2
GM	533 ± 5	39 ± 45	8[e]	28 ± 17	74	226 ± 19	292 ± 19	89 ± 47	4 ± 600	45[f]	16 ± 600	258 ± 78	980 ± 162	803 ± 40	200[d]	115 ± 35	29
Fistula x̄	445	41	10	10	61	284	218	306	419	64	–[g]	–[g]	315	349	–[g]	36	–[g]
GAIII	287 ± 4	7 ± 35	1 ± 52	<1 ± 275	8	238 ± 5	77 ± 6	120 ± 6	670 ± 16	54 ± 6	13 ± 170	12 ± 310	21 ± 35	113 ± 77	386 ± 12	7 ± 29	7
DB	223 ± 8	15 ± 9	2 ± 9	4 ± 17	20	268 ± 13	119 ± 16	58 ± 26	364 ± 43	23 ± 33	107 ± 150	189 ± 115	7 ± 400	10 ± 600	200[d]	29 ± 8	7
GAII	275 ± 5	19 ± 14	<1 ± 64	3 ± 31	22	202 ± 20	82 ± 13	89 ± 24	<1 ± 220	1 ± 270	982 ± 23	999 ± 26	308 ± 66	10 ± 600	200[d]	29 ± 5	7
EHII	223 ± 8	43 ± 13	4[e]	10 ± 13	57	210 ± 39	86 ± 26	87 ± 44	<1 ± 140	21[f]	483 ± 38	672 ± 33	26 ± 49	10 ± 600	200[d]	38 ± 5	10
MV	451 ± 6	35 ± 9	4[e]	14 ± 20	53	196 ± 17	74 ± 20	74 ± 48	<1 ± 600	20[f]	4 ± 535	1 ± 600	12 ± 108	600 ± 38	200[d]	22 ± 50	5
WR	256 ± 17	23 ± 24	2[e]	5 ± 33	30	173 ± 55	101 ± 31	120 ± 47	3,949 ± 102	17[f]	<1 ± 600	11 ± 600	12 ± 315	2,200 ± 200	200[d]	9 ± 400	2
HR	391 ± 8	19 ± 47	2[e]	1 ± 195	22	593 ± 38	208 ± 17	180 ± 66	2,965 ± 156	59[f]	12 ± 600	232 ± 260	170 ± 110	714 ± 460	200[d]	23 ± 200	6
Normal x̄	301	23	2	5	30	269	107	104	–[g]	28	–[g]	–[g]	80	–[g]	–[g]	22	–[g]
FH-A	584 ± 10	12 ± 14	<1 ± 86	<1 ± 260	12	1274 ± 11	116 ± 11	135 ± 16	<1 ± 600	80 ± 16	<1 ± 600	246 ± 305	35 ± 50	923 ± 13	157 ± 470	<1 ± 600	<1

Rate constant values shown are $min^{-1} \times 10^5 \pm FSD \times 10^2$. FSD, fractional standard deviation (SD ÷ value).

[a] L(β,5) = L(7,5) + L(10,5) + L(8,5).

[b] L(7,17) = 1.667 × L(17,7), forcing M(17) to equal 60% of M(7).

[c] L(6,7) was fairly well defined (FSD ~ 40%) in SW and GAIII. L(6,7) was marginal or undefined and usually ~25% of L(12,7) in all other subjects. Therefore, L(6,7) = 0.25 × L(12,7) in all subjects except SW and GAIII.

[d] L(12,10) was fixed at 0.002; IDL was not separated from LDL in most of these subjects, and L(12,10) was marginal or undefined (FSD > 80%) when free to adjust. L(12,10) = 0.002 based on results of sensitivity testing of multiple studies and unpublished data.

[e] IDL was not separated from LDL; L(10,5) = 0.2 × L(7,5) in EHI and GM; L(10,5) = 0.1 × L(7,5) in EHII, MV, WR, and HR based on subjects with IDL in the group.

[f] IDL was not separated from LDL; L(10,9) = 0.2 × L(7,9) in EHI and GM; L(10,9) = 0.1 × L(7,9) in EHII, MV, WR, and HR based on subjects with IDL in the group.

[g] Mean is not shown because most values were undefined (FSD > 80%) or fixed.

TABLE 4. Transports and pool sizes derived from compartmental analysis with the final model

Subject	R(9,4)	R(β,5)[a]	R(7,9)	R(9,7)	R(8,9)	R(9,8)	R(10,9)	R(10,8)	R(7,10)	R(17,7); R(7,17)	R(12,8)	R(12,10)	R(12,8) + R(12,10)	R(6,7) + R(12,7)	M(12)	M(6)
SW	4.15	3.85	12.19	11.77	12.73	12.42	3.42	1.00	0.67	11.81	0.00	4.46	4.46	3.54	1,520 ± 13	35,952 ± 17
ID	2.77	0.74	3.12	3.94	5.01	1.89	0.48	0.80	0.31	1.52	2.32	1.14	3.46	0.06	4,636 ± 5	43,089 ± 4
EB	2.85	1.21	5.19	6.82	6.83	3.50	1.14	0.42	0.63	0.91	2.97	1.08	4.05	0.01	5,909 ± 5	33,248 ± 5
EHI	1.96	0.86	5.22	7.97	6.36	2.70	1.04	2.34	2.39	7.86	1.32	1.14	2.46	0.36	3,174 ± 6	71,215 ± 4
GM	3.90	0.81	5.00	4.06	1.97	0.01	1.00	0.04	0.64	13.64	2.22	0.49	2.72	2.00	6,258 ± 4	53,176 ± 3
Fistula x̄	3.13	1.49	6.14	6.91	6.58	4.10	1.42	–[b]	–[b]	7.15	1.77	–[b]	3.43	1.19	4,299	47,336
GAIII	2.53	0.25	5.87	5.14	2.96	2.50	1.33	0.05	0.04	1.41	0.42	1.36	1.78	1.00	1,320 ± 10	20,911 ± 6
DB	2.47	0.61	9.02	7.71	1.95	1.56	0.77	0.46	0.63	0.47	0.04	0.66	0.71	2.37	6,362 ± 7	20,478 ± 11
GAII	2.45	0.79	6.00	6.23	2.64	0.00	0.04	2.72	2.33	23.29	0.03	0.43	0.46	2.78	6,709 ± 13	22,007 ± 22
EHII	1.47	1.31	3.65	4.04	1.51	0.00	0.36	1.69	1.66	1.22	0.04	0.49	0.53	2.26	3,877 ± 8	32,075 ± 6
MV	3.46	1.42	5.58	4.78	2.10	0.00	0.56	0.02	0.00	0.77	2.46	0.66	3.12	1.75	5,947 ± 6	38,997 ± 17
WR	2.16	0.64	5.95	5.79	4.11	2.71	0.59	0.00	0.03	0.69	1.51	0.61	2.12	0.68	7,326 ± 9	22,459 ± 31
HR	2.96	0.57	14.66	14.02	4.45	3.60	1.47	0.01	0.82	11.46	0.87	0.71	1.58	1.96	2,633 ± 10	12,406 ± 18
Normal x̄	2.50	0.80	7.25	6.81	2.82	–[b]	0.73	–[b]	–[b]	5.62	–[b]	–[b]	1.47	1.83	4,882	24,191
FH-A	2.66	1.23	29.45	31.76	3.11	0.00	1.86	0.00	1.14	9.56	3.12	0.73	3.85	0.04	7,913 ± 9	22,467 ± 43

Transport values are μmol/min/70 kg, and pool size values are μmol/70 kg ± FSD × 10^2. M(3), M(4), M(5), M(7), M(8), M(9), and M(10) are not shown. They were all 97–103% of the concentration (Table 2) × 31.5 dl (plasma volume as 4.5% of body weight in kilograms, normalized to 70 kg). In EHI and GM, IDL was not separated from LDL and M(10) was assumed to constitute 15% and M(7) 85% of the combined concentration in Table 2 based on subjects with IDL in the fistula group; in EHII, MV, WR, and HR, based on subjects with IDL in the normal group, M(10) was 5% and M(7) was 95% of that in Table 2. M(17) equaled 60% of M(7).

[a] R(β,5) = R(7,5) + R(10,5) + R(8,5).

[b] Mean not shown because most corresponding rate constants were undefined (FSD > 80%; Table 3) or fixed.

ester was produced from HDL free cholesterol and 30% from β-lipoprotein free cholesterol. Total ester production in subject FH-A was 18% above normal, and bile fistula subjects produced 40% more than normal.

L(7,9) and L(9,7) represent cholesteryl ester transfer between HDL and LDL and were well defined in all 13 subjects. R(7,9) and R(9,7) (Table 4) were nearly equal within each subject, showing considerable exchange as reflected by the strongly positive linear correlation, R(7,9) = 0.93 × R(9,7) + 0.39 (r^2 = 0.97; n = 13). This neither proves nor excludes net transfer of ester to LDL from HDL in vivo. To detect net transfer in the presence of considerable exchange, all other pathways in the ester part of the model should be very well defined (FSD < 20%). In the two subjects closest to this goal, GAIII and SW, R(7,9) slightly exceeded R(9,7).

L(8,9) and L(9,8) represent ester transfer between HDL and VLDL. L(8,9) was well defined (FSD < 50%) in 12 subjects. L(9,8) was well defined in five subjects. R(8,9) exceeded R(9,8) in every subject, as reflected by the positive intercept (95% confidence interval of 1.4–3.1 μmol/min/70 kg) of their linear correlation, R(8,9) = 0.86 × R(9,8) + 2.25 (r^2 = 0.87; n = 13). This shows net transfer of ester to VLDL from HDL along with some exchange. There was a fair correlation (r^2 = 0.68, n = 13) between R(8,9) and M(8) but a poor correlation (r^2 = 0.58, n = 13) between R(9,8) and M(8). L(8,9), R(8,9), and M(8) were 2- to 3-fold higher in bile fistula than in FH-A and normal subjects.

L(10,9) and L(10,8) represent pathways by which cholesteryl ester can transfer to IDL. L(10,9) was well defined in six of the seven subjects with IDL isolated, whereas L(10,8) was well defined in only one of the seven. R(10,9) generally exceeded R(10,8). L(7,10) represents ester transfer to LDL from IDL. L(7,10) was well defined in only one of the seven subjects with IDL isolated. L(10,8) and L(7,10) represent the conventional concept of ester movement in the particle core to LDL from VLDL, yet they were poorly defined in most subjects and the transports [R(10,8) and R(7,10)] were of low magnitude.

L(12,8), L(12,10), L(12,7), and L(6,7) represent the cholesteryl ester output pathways in the final model (Fig. 6, dashed arrows). They were generally not as well defined, as shown by larger FSDs, as other pathways in Table 3, but they revealed three trends. First, ester output to extrahepatic tissue [C(6)] was small compared with output to hepatic tissue [C(12)]. Second, L(12,8) was better defined than L(12,7) in FH-A and most fistula subjects, whereas L(12,7) was better defined than L(12,8) in most normal subjects. Third, in FH-A and all fistula subjects, ester output was greater from VLDL plus IDL [Table 4; R(12,8) + R(12,10)] than from LDL [R(6,7) + R(12,7)]. In normal subjects, there was slightly more output from LDL than from VLDL plus IDL. Thus, most ester output is to the liver from the gamut of apolipoprotein B (apoB)-containing particles, and regulation may occur at different sites therein.

DISCUSSION

The model shown in Fig. 6 is the first in humans to identify and quantify the major in vivo pathways of cholesteryl ester transport to and from all lipoproteins. Among many findings were the importance of both α- and β-activities of LCAT in plasma ester production, net transfer of ester to VLDL and IDL from HDL, the presence of considerable bidirectional exchange of ester between LDL and HDL, and output of ester to the liver from VLDL, IDL, and LDL. Notably absent in normal subjects was output of cholesteryl ester to tissues from HDL. Furthermore, output of ester to tissue from HDL was not detectable in subjects with FH and bile fistula, in whom its upregulation might be anticipated.

Results in all subjects showed the traditional α-activity of LCAT on HDL and β-activity of LCAT using LDL free cholesterol as substrate, as found in vitro (23). In the majority of the 13 subjects there was evidence for β-activity of LCAT on both IDL [L(10,5)] and VLDL [L(8,5)] using β-lipoprotein free cholesterol specific activity as an approximation for each. In three other subjects, administered free [^{3}H]cholesterol or [^{14}C]cholesterol in VLDL, there was support for LCAT activity on VLDL (see Results). In vitro studies have yielded positive and negative results (23–26). An unequivocal conclusion that LCAT can use VLDL and IDL in vivo awaits methods for their rapid isolation to prevent ex vivo free cholesterol exchange.

Total production of cholesteryl ester in normal subjects was 3.3 μmol/min/70 kg [Table 4; R(9,4) + R(β,5)], equivalent to 63 μmol/h/l. In vitro production was similar at ∼90 μmol/h/l, as summarized by Dobiasova (27). The rate constant for β-LCAT was 0.00030 min^{-1}, which was 10% of that for α-LCAT in normal subjects (Table 3). Because the mass of free cholesterol was greater in apoB lipoproteins than in HDL, β-LCAT produced ∼30% of plasma ester, a substantial portion that is compatible with its role in fish-eye disease (2, 24). Both α- and β-activities of LCAT were increased by ⩾48% in bile fistula subjects. A similar increase was reported during colestipol therapy (28), a perturbation analogous to bile fistula.

Two negative findings regarding cholesteryl ester production are of note. First, no evidence was found for tissue-produced ester in plasma. This contrasts with findings in the rat, in which VLDL is assembled and secreted with hepatic ACAT-produced cholesteryl ester (29). However, ACAT activity is high in rat liver but low in humans (29). In addition, Rudel and coworkers (30) have suggested that ACAT-2 participates in lipoprotein assembly, and there is relatively little ACAT-2 in human liver (31). This negative finding was rigorously tested in the four subjects simultaneously administered [^{14}C]mevalonic acid to initially label hepatic cholesterol and free [^{3}H]cholesterol in HDL or LDL to initially label plasma; when β-LCAT activity on VLDL [L(8,5)] was removed from the model and a pathway to VLDL from the liver [L(8,12)] was added, the simulations of [^{14}C]VLDL and [^{3}H]VLDL ester observations were dissociated during the initial 100 min. Hepatic subcompartments, a delay for hepatic assembly, or a plasma VLDL delipidation chain did not improve the dissociation. In the presence of β-LCAT activity on VLDL [L(8,5)], a small (⩽0.1 μmol/min/70 kg) ester contribution to VLDL from the liver could not be excluded. This could explain why ester is not completely absent in the plasma of subjects with classic LCAT deficiency (1, 2), as could a contribution from the intestine after meals.

Second, free cholesterol that effluxes to acceptors from extrahepatic tissues [C(25) and C(6)] was not esterified before mixing with plasma HDL free cholesterol [C(4)]. In the three subjects administered particulate [^{14}C]cholesterol, the ^{14}C activity of cholesterol leaving C(25) [probably reticuloendothelial cells (32)] during the initial 8 h was much higher than any other pool. Esterification of this "hot" cholesterol on its acceptor, then transfer to the HDL ester pool [L(9,25)], to another ester pool [such as L(7,25)], or directly to liver/bile [L(12,25)], would be easily detected. The results show that the acceptor carried all [^{14}C]cholesterol to plasma HDL [L(4,25) in Fig. 6]. Cholesterol on acceptors from tissues not detected herein could be carried elsewhere or esterified directly, but no clues were found for these possibilities.

There was vigorous bidirectional exchange of cholesteryl ester between HDL and LDL, but no evidence for net transport. This agrees closely with the conclusion and transport values from in vitro experiments by Barter and Jones (8). No physiologic role for this putative CETP-mediated futile cycle is apparent, but net transport could occur during periods of nonsteady state. The exchange of ester between HDL and LDL was increased 5-fold in the subject with FH, probably because of increased CETP (33, 34).

There was a small component of bidirectional exchange of cholesteryl ester between HDL and VLDL. In addition, in all 13 subjects there was net transport to VLDL from HDL, possibly by CETP-mediated exchange for VLDL triglyceride (5, 7, 9); the latter could account for the correlation between VLDL pool size [M(8)] and ester transport to VLDL from HDL [R(8,9)]. The total transport of ester to VLDL from HDL in normal subjects [R(8,9) of 2.82 μmol/min/70 kg; Table 4] was only 35% higher than the in vitro rate reported by Guerin et al. (35).

Cholesteryl ester transport to IDL and LDL from HDL exceeded the transport of core-locked ester to IDL from VLDL and to LDL from IDL. This shows the dynamic nature of cholesteryl ester in human blood first proposed by Zilversmit and colleagues (4) and later attributed to CETP (5). Our findings (Fig. 6) also support in vitro data showing that LTIP may inhibit specific ester transfers (9). For example, we found no ester transfer to VLDL from LDL.

No evidence was found for cholesteryl ester output to tissue directly from HDL as pathway L(12,9) or L(6,9) in Fig. 1. When fixed to a range of values, they could exist combined up to 0.4 μmol/min/70 kg with minimal (⩽2%) increase in the sum of squares but above which the sum of squares increased rapidly. Recently, we administered [^{3}H]cholesteryl linoleate in HDL (36), a more severe test for the pathways. Preliminary kinetic analysis showed ester output of ∼0.3 μmol/min/70 kg to tissue from HDL, accounting for 10% of total ester output. Thus, neither selective nor holoparticle ester output from HDL was detectable with the current design, but it probably occurs to a minor degree in humans.

No evidence for extravascular HDL cholesteryl ester was found. Because kinetic studies in humans have found large extravascular pools of apoA-I and apoA-II (37), they may represent lipid-poor apolipoproteins outside the circulation. Extravascular LDL ester was found with kinetics comparable to those of extravascular apoB (38), suggesting that LDL particles may exit and reenter the circulation; this could represent reversible binding to proteoglycans or retroendocytosis.

Cholesteryl ester kinetics in normal subjects revealed two additional similarities with apoB kinetics. First, the irreversible output of ester from LDL [L(12,7) + L(6,7)] averaged 0.00028 min^{-1}, which is 0.4 d^{-1}, the same as

LDL apoB output per day (38, 39). Second, 30–50% of apoB output is from VLDL/IDL and 50–70% is from LDL (40–44). Ester output was harmonious: 44% from VLDL/IDL [R(12,8) + R(12,10) in Table 4] and 56% from LDL [R(6,7) + R(12,7)]. The convergence of apoB and ester outputs implicates holoparticle uptake by the LDL receptor family in basal reverse cholesterol transport.

The five bile fistula subjects in our study represent a paradigm for upregulated reverse transport. Cholesteryl ester output from VLDL/IDL was more than double that of normal subjects (3.43 vs. 1.47 μmol/min/70 kg; Table 4) and constituted 74% of total ester output versus 44% normally. Ester production [R(9,4) + R(β,5); Table 4] was 40% higher in fistula than in normal subjects. There was neither a change in most transports that may involve CETP nor detectable HDL ester output in fistula subjects. However speculative, these results implicate LCAT and the LDL receptor family in the upregulation of reverse transport.

Results in FH contrasted with normal subjects in three respects. First, rate constants for irreversible output of LDL cholesteryl ester [L(12,7) and L(6,7)] were extremely low, compatible with the absence of LDL receptors. This accounts for the failure of plasma esterified and free cholesterol specific activities to approach each other (Fig. 4). Second, total ester production in FH-A was on the high side of normal [R(9,4) + R(β,5); Table 4]; this was balanced by the highest output to the liver from VLDL [R(12,8)] of the 13 subjects. These observations are compatible with the regulation of reverse transport via LCAT and the LDL receptor family. Third, the enhanced exchange of ester between HDL and LDL in FH-A explains the normal equilibration of their specific activities (Fig. 4) but leaves unresolved the role of CETP in the regulation of reverse transport.

Free cholesterol transport in blood and liver was examined, and no major difference between FH, normal, and bile fistula subjects was found. HDL free cholesterol [C(4)] was very dynamic, with a half-life of 7–9 min. There was no evidence for hepatic subcompartments or channeling of cholesterol to bile from synthesis, LDL, IDL, VLDL, or HDL, in agreement with previous human studies (13). As shown in Fig. 6, where extrahepatic synthesis [U(6)] is zero, there was net transport of free cholesterol (1.2 μmol/min) to the liver from HDL, accounting for 29% of the total plasma-to-liver reverse transport. The total of 4.14 μmol/min includes esters from VLDL, IDL, and LDL plus net free cholesterol from HDL. To test the influence of extrahepatic synthesis on transport in normal subjects, U(6) was fixed at 1.4 μmol/min/70 kg and U(1) at 0.7 μmol/min/70 kg, keeping the total at 2.1 μmol/min/70 kg. Results showed that all transports involving cholesteryl ester were unchanged; efflux to HDL [R(4,6)] increased by 1.4 mol/min/70 kg, and transport to the liver from HDL [R(12,4)] increased by 1.0 μmol/min/70 kg over those in Fig. 6; R(5,12) decreased by 0.4 μmol/min/70 kg, and other changes were less than 0.2 μmol/min/70 kg. However conjectural, this approach shows that cholesterol synthesized outside the liver would efflux to HDL and increase net free cholesterol transport to the liver from HDL to as much as 41% of the total. By analogy, surplus cholesterol could efflux to HDL by upregulation of ABCA1 or infusion of apoA-I complexes and increase net free cholesterol transport to the liver. Scavenger receptor class B type I (SR-BI) could facilitate this HDL free cholesterol transport to the liver (45).

Many of our results are relevant to the transport of cholesterol from peripheral tissue to the liver, so-called reverse transport. The major pathways identified were efflux to HDL, free cholesterol output to the liver from HDL as well as esterification in HDL, transfer of the esters to VLDL and IDL, then output to the liver from VLDL, IDL, and LDL, probably by particle uptake. If a perturbation occurs, it is likely that the pathway(s) enlisted would depend on the paradigm: esterification and VLDL plus IDL ester output increased in subjects with a bile fistula, and VLDL ester output increased in the FH subject. We hypothesize that esterification and VLDL ester output would decrease with statin therapy and that HDL free cholesterol output to liver would increase as tissue efflux increases.

The paucity of cholesteryl ester output to liver from HDL in humans is in stark contrast to that in mice (no CETP), in which HDL ester output is major (46). In rabbits (high CETP), output of ester is more like that of humans, with 70% from VLDL plus LDL and 30% from HDL (47). Of note, HDL ester output to a small tissue (adrenal, ovary, etc.) might easily be missed by our analysis of large pathways. We have no explanation for the paucity of in vivo selective uptake of HDL ester by the human liver, which contains SR-BI (11, 12). The paucity implies that the search for SR-BI-deficient humans may not be fruitful by screening based on very high HDL.

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