In vivo cholesterol kinetics in apolipoprotein E-deficient and control mice

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Abstract The in vivo total body cholesterol transport of homozygous apoE-deficient ($-/-$) and control ($+/+$) mice was evaluated by compartmental analysis of plasma cholesterol decay. Body cholesterol fractional catabolic rates of chow fed mutants were less ($-/-$, 0.17 ± 0.02; $+/+$, 0.51 ± 0.06 day^{-1}) and body cholesterol contents greater ($-/-$, 68 ± 5; $+/+$, 48 ± 5 μmol) than controls. The body cholesterol expansion of the chow-fed mutant was extracellular with at least half in plasma. Cholesterol transport, i.e., the mass entering, moving through, and exiting the body each day, was similar ($-/-$, 6.9 ± 0.7; $+/+$, 8.5 ± 0.9 μmol/day) for homozygotes and controls on chow, and both tripled with cholesterol feeding. Differing from controls, however, mutants had considerable expansions of plasma and body cholesterol ($-/-$, 166 ± 21; $+/+$, 59 ± 11 μmol) with increments in peripheral tissue cholesterol contents. Cholesterol feeding increased control hepatic cholesterol without a change in plasma, whereas mutants had large increments in plasma cholesterol with no change in liver. Consistent with impaired hepatic uptake of cholesterol, mutants had much slower plasma clearance of lipoprotein cholesterol, as well as slower transfer to catabolic pools than normals. Treatment of homozygotes with lovastatin doubled both plasma cholesterol concentration and body cholesterol transport indicating the importance of apoE-dependent cell cholesterol transfer in synthetic down-regulation with this agent. ■ These data indicate that mice lacking apoE have lower affinity hepatic uptake of plasma remnant cholesterol. This is an important factor in producing a lower total body cholesterol fractional catabolic rate which results in a considerable increase in body cholesterol mass to achieve transport capacities equivalent to mice with apoE.—Quarfordt, S. H., B. Oswald, B. Landis, H. S. Xu, S. H. Zhang, and N. Maeda. In vivo cholesterol kinetics in apolipoprotein E-deficient and control mice. *J. Lipid Res.* 1995. **36:** 1227–1235.

Supplementary key words apoE-deficient mutant • cholesterol transport • fractional catabolic rate • lovastatin

The absence of apoE changes the access of plasma lipoprotein cholesterol to cells in mice with this deletion. Rather than the characterized (1) remnant receptor, possibly low density lipoprotein related protein (2) and appropriate proteoglycans (3) that bind apoE, chylomicron and very low density lipoprotein (VLDL) remnant cholesterol enter hepatocytes by an apoE-independent system. The small amount of mouse apoB-100 on low density lipoproteins (LDL) and VLDL (4), and the greater affinities of apoE for the LDL receptor (5), would also limit cell cholesterol uptake by this receptor.

The mutants lacking apoE move cholesterol from plasma into cells by uncharacterized pathways obviously independent of apoE. At present, we have little understanding of the properties of such pathways other than the observation that remnant-like lipoproteins accumulate in the mutant plasma even on standard chow diets (6, 7). This suggests that apoE-independent clearance has lower affinity and possibly even lower capacity than that dependent on apoE. Other properties of apoE-independent plasma lipoprotein cholesterol transport, including molecular mechanisms, are undefined.

Aside from altering plasma lipoprotein cholesterol transport, the lack of apoE quite likely influences total body cholesterol homeostasis. Any impairment in providing plasma cholesterol to cells, particularly hepatocytes (8), could up-regulate synthesis and increase total body transport of cholesterol. Such an occurrence could exacerbate the plasma hypercholesterolemia and, depending on catabolism, increase whole body cholesterol mass. Secondly, apoE-independent transport may result in a catabolic defect manifested by a lower body cholesterol fractional catabolic rate and a larger whole body cholesterol mass than controls. As remnant transport has been proposed (9) as a major vehicle for reverse cholesterol transport, any limitation in this pathway may influence catabolism. Finally, although the mutants have major increments in plasma cholesterol, this may simply reflect an altered distribution of body cholesterol. Total body

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cholesterol mass, the transport of cholesterol through the body, and body cholesterol fractional catabolic rate may be the same for mutant and control.

The studies presented here determine parameters of in vivo cholesterol transport in homozygous apoE-deficient mice and their control littermates. The studies were done on mice fed standard chow where synthesis was the predominant input. Other studies evaluated effects of lovastatin-induced alterations in synthesis in control and mutant mice. Finally, cholesterol transport was enhanced in both types of mice by cholesterol feeding.

MATERIALS AND METHODS

Materials

[1,2-³H]cholesterol and [23-¹⁴C]taurocholate were purchased from DuPont New England Nuclear (Boston, MA). [4-¹⁴C]cholesterol came from Amersham (Chicago, IL). Egg phosphatidylcholine was from Avanti (Pel, AL) and cholesterol from Alltech (Deerpark, IL). The tracer and cold cholesterol were determined to be pure (> 99%) by both adsorptive and reverse-phase thin-layer chromatographic systems. Organic solvents were obtained from Mallinckrodt (St. Louis, MO) and were analytic grade. Lard was obtained from Fischer (Louisville, KY) and dietary cholesterol from ICN (Aurora, OH). Lovastatin was a generous gift from Merck, Sharpe and Dohme (Rahway, NJ).

Animals

Control mice were normal littermates of the mutant mice with apoE deletions (10). Males and females ranging in weight from 15 to 36 g and in age from 2 to 10 months were used. On a standard rodent chow diet the mice with homozygote deletions had body weights similar to those of control mice and all had plasma cholesterol levels at least fourfold the control. All mice were maintained on either standard rodent chow 3000 (Agway; Syracuse, NY containing 0.01% cholesterol by gas–liquid chromatography) or chow supplemented with 10% lard and made to 1% cholesterol, or chow containing 0.025% lovastatin. They were fed for at least 3 weeks prior to study. Isotopic studies were initiated between 8 and 10 AM. Tracers were injected in the tail vein and blood was obtained from the retroorbital plexus.

Cannulation of the bile duct in both control and mutant mice was achieved with Tygon tubing (0.25 mm ID, 0.75 mm OD) (Norton Co., Akron, OH) at the level of the mid common duct after 1 mg intraperitoneal pentobarbital anesthesia. A cannula was also placed in the mid duodenum. Both cannulas were tunneled to the dorsal cervical area where they were connected with PE 60 tubing (Becton Dickinson, Parsippany, NJ) to maintain an intact enterohepatic circulation, an important deter-

minant (11) of the precursor source of biliary cholesterol and bile acid. The day after surgery the bile flows of the mice were measured and those less than 50 μl/h were excluded from study. The remaining mice were injected with apoE-deficient homozygote serum containing 0.1 μCi, 1.4 μmol [1,2-³H]cholesterol-labeled lipoproteins that had been harvested from mutants injected with free cholesterol in phosphatidylcholine the previous day and determined to have more than 65% of the tracer in cholesteryl ester. Serum was obtained at intervals and, after ultracentrifugation, was assayed for tracer distribution in both the d 1.063 g/ml top and bottom and in free and esterified cholesterol. Bile was collected for 2-h intervals during the day and two-thirds of the bile was returned to the duodenum. The cannulas were connected throughout the night and reopened the following morning and similar aliquots were obtained. At the conclusion of the study [23-¹⁴C]taurocholate (0.1 μCi) was put in the intestine and bile samples were obtained 4–8 h later to determine pool size by an isotope dilution method (12).

To evaluate in vivo cholesterol kinetics, tracer (3–6 μCi) free cholesterol (10 μg) was injected as a 4/1 molar ratio of egg phosphatidylcholine/free cholesterol in 0.5 ml of phosphate (0.2 M, pH 7.4)-buffered saline (0.13 M). All injections were in the tail vein and blood was obtained from the retroorbital plexus. Early (5–120 min) blood sampling of both controls and homozygotes established that 30 min was required for the initial exponential of decay to appear. The first blood sample (50 μl) was obtained at that time, then daily for 4 days, and biweekly out to 3 or 4 weeks. At the conclusion of the study, the mice were exsanguinated and the tissues were extracted and assayed.

Analytic methods

Free and esterified cholesterol was extracted from plasma and tissue by the Dole method (13). Determinations of cholesterol mass were by a cholesterol oxidase method using commercial kits for both free and esterified cholesterol (Wako, Osaka, Japan). The mouse plasma was made to d 1.063 g/ml and ultracentrifuged (1.8 × 10⁸ g min) in a Beckman Model L5-75 centrifuge (Palo Alto, CA) to separate lower and higher density lipoproteins (14). Tracer free and esterified cholesterol were isolated by thin-layer chromatography on silica gel G with the solvent system petroleum ether–diethyl ether–glacial acetic acid 80:20:1. Biliary cholesterol was separated from the bile acids by a chloroform–methanol 2:1 (20 vol/vol bile) extraction. After the phases were split with 5 vols 0.05% H_2SO_4, the chloroform phase was taken for cholesterol mass and radioactivity assay, and the methanol phase for similar assays of bile acids. Bile acid mass was determined by a 3α-steroid dehydrogenase method (15). Bile acids were radioassayed in an aqueous scintillation cocktail 3a70B (Research Products, Mt. Prospect, IL) and cholesterol in 0.5% 2,5 diphenyloxazole, 0.01% phenylox-
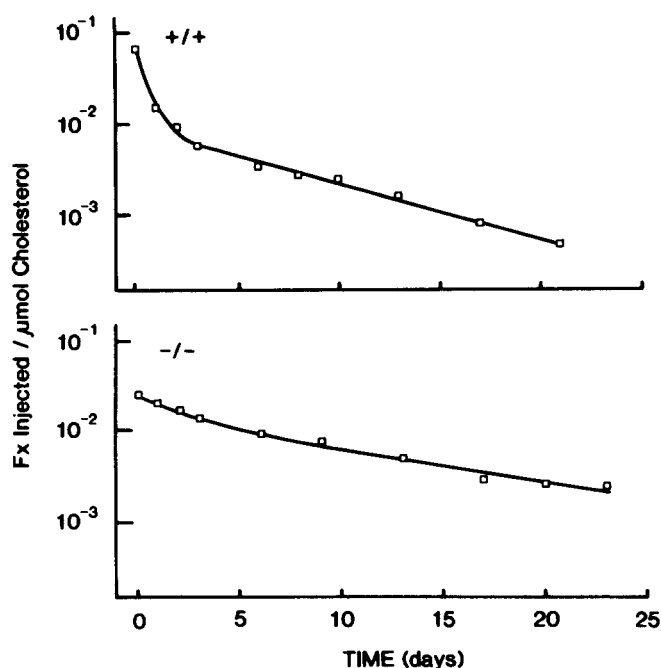
Fig. 1. Plasma cholesterol decay in chow-fed normal and homozygote apoE-deficient mice. The data are representative plasma cholesterol specific activity functions from normals (+/+, top panel) and homozygotes (−/−, bottom panel) after the injection of [1,2-^{3}H]cholesterol. The curves are the simulations using the two-pool model from the experimental data points presented.

azolylbenzene in toluene. Radioassay was in a Beckman (Palo Alto, CA) model 8100 liquid scintillation spectrophotometer.

Calculations

All isotopic data were expressed as the fraction of injected activity per μmol of cholesterol. The biexponential plasma decay data were simulated by a two-compartment model previously used (16) in the analysis of similar human data using the program SAAM 31 (17). Pool 1, or the pool into which the isotope was introduced, was termed the rapid pool (R) and the second pool was termed the slow pool (S). These two pool models assume all input into and catabolism from the rapid turnover pool. This catabolic assumption appears justified in humans (16).

Only studies demonstrating satisfactory simulation of the experimental data and precise determinations (fractional standard deviation < 12%) of the three fractional rate constants were included in the data. Three studies of chow-fed homozygotes and two of controls were not included because one or more parameters were insufficiently precise. The homozygotes and control studies that were not included demonstrated the same respective properties as those presented. The mass of the injection compartment (pool 1 or R) was determined by the program from the fit of the data and the total mass in the body, and the cholesterol transport through the body was calculated from these data, using the three fractional rate constants obtained from the function. Body cholesterol transport was the product of the mass in the rapid pool (R) and the total body fractional catabolic rate, both of which were the best determined (FSD < 5%) of all the parameters. This value differs from plasma transport that was the product of the fractional plasma clearance rate and the vascular cholesterol pool. Plasma cholesterol transport was only quantitated in the mutant. Body cholesterol input indicates new cholesterol entering the body. Body cholesterol transport, as defined here, is that fraction of total transport destined for catabolism and was calculated as described above. In steady state conditions, input, body cholesterol transport, and cholesterol-derived compounds excreted are the same. Significance determinations were obtained with a two-tailed Student's t test or by repeated measures of analysis of variance (18).

RESULTS

When normal animals were fed regular chow, the plasma decay of tracer cholesterol had a distinctly more rapid initial than terminal exponential (**Fig. 1**). In the homozygous apoE-deficient mutants, on the other hand, the slopes of the initial and terminal exponential were similar, and the integral of the function was considerably larger than normal (Fig. 1). The compartmental analyses of these data, using a two-pool system with catabolism solely from the rapid pool (R) in equilibrium with a slower turning over pool (S), indicate that the total body

TABLE 1. In vivo cholesterol transport in chow-fed normal and apoE-deficient homozygote mice

	Fractional Transfer Rates Day^{-1}			Pool Size μmol		Transport	
	R→S	S→R	FCR	R	Total	μmol/day	μmol/day/10 g
+/+, n = 6	0.95 ± 0.05	0.55 ± 0.07	0.51 ± 0.06 (0.29–0.72)	17 ± 3	48 ± 5 (33–55)	8.5 ± 0.9	2.9 ± 0.7
−/−, n = 5	0.29 ± 0.01[c]	0.52 ± 0.11	0.17 ± 0.02[b] (0.12–0.20)	41 ± 2[c]	68 ± 5[a] (57–82)	6.9 ± .7	2.8 ± 0.3

Data represent the mean ± SE of the number of studies indicated. The mean weight ± SE of the mutants were 29 ± 3 and of controls 25 ± 3 g. The values within parentheses are the ranges for these parameters.
Significantly different from control at [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

TABLE 2. Tissue cholesterol mass in chow- and cholesterol-fed normal and apoE-deficient heterozygotes and homozygotes

	Mass [% Free]			
	Chow-Fed		Chol-Fed	
Tissue	+/+ (n = 5)	-/- (n = 4)	+/+ (n = 5)	-/- (n = 4)
	$\mu mol/g$ (w/w) ± SE			
Liver	3.6 ± 0.8(59)	3.3 ± 0.5(69)	7.2 ± 0.5(43)	1.8 ± 1.0(75)
Spleen	4.9 ± 0.3(63)	5.9 ± 0.3(83)	8.2 ± 1.5(69)	10.8 ± 1.3(50)[a]
Kidney	8.3 ± 1.8(66)	6.4 ± 0.3(64) .	6.9 ± 0.8(69)	9.5 ± 0.5(56)[b]
Muscle	4.4 ± 1.0(71)	2.8 ± 0.8(54)	3.1 ± 0.5(77)	4.4 ± 0.8(41)
Adipose	5.7 ± 1.0(82)	5.4 ± 0.8(62)	3.9 ± 0.5(81)	9.8 ± 0.8(27)[c]
Heart	4.6 ± 0.8(67)	6.2 ± 0.8(62)	4.4 ± 0.5(83)	9.5 ± 0.5(41)[c]
Lung	7.7 ± 1.3(67)	11.3 ± 2.3(68)	8.0 ± 0.8(66)	16.2 ± 1.8(62)
Skin	8.2 ± 2.3(66)	5.9 ± 1.5(65)	6.9 ± 1.5(70)	13.4 ± 1.8(52)[a]
Serum	2.4 ± 0.1(71)	12.3 ± 0.3(68)[b]	2.8 ± 0.3(66)	27.8 ± 2.1(62)[b]

The data are the means of the number of mice indicated, assayed at the conclusion of the cholesterol turnover study (Table 1). Data within parentheses are the percent free cholesterol.

Significantly different from chow controls or respective chow homozygote at [a]$P < 0.01$ or [b]$P < .001$.

[c]The percent of free cholesterol is significantly less than respective control $P < 0.05$.

cholesterol fractional catabolic rate in the homozygotes is one-third of that in normal mice (**Table 1**). The fractional transfer from R to S is considerably slower for the homozygotes than for the normal mice. The fractional transfer from S to R, the size of S, and the cholesterol transport between pools in homozygotes fed chow are equivalent to that in normals. This indicates that the lack of apoE influences the effectiveness of cholesterol transfer from pool R to S but not out of S.

Despite the smaller body cholesterol fractional catabolic rate, the total transport of cholesterol through the body of homozygotes fed chow is the same as normal. This is because the size of R is increased in homozygotes. While about two-thirds of body cholesterol in normal mice is in the slower turning over pool, the major portion of body cholesterol in homozygotes is in the rapid turnover pool. Total body cholesterol is significantly larger in the mutant and this cholesterol increment was confined to the rapid turnover pool. Plasma cholesterol is an appreciable component of this pool and the large difference in plasma cholesterol between mutants and control mice (**Table 2**) was estimated to account for at least half of the mutant increment in pool R and body cholesterol. The cholesterol contents and ratios between free cholesterol and cholesteryl ester in various tissues of chow-fed normals and homozygotes are not significantly different (Table 2). As these mice were fed chow, with a cholesterol intake of 0.01% of total food intake or 1.2 μmol of cholesterol (assuming total absorption), cholesterol input was more than 80% synthetic. This similarity in synthetic input in the mutants and normals suggests either that apoE-independent cholesterol entry into cells regulates synthesis as effectively as does apoE-dependent cholesterol uptake, or that cellular cholesterol synthesis is

primarily regulated by other pathways (i.e., B-100, etc.).

Feeding a diet containing 1% cholesterol for a month prior to study considerably influenced the plasma decay of cholesterol in both normal and apoE-deficient mice (**Fig. 2**). In comparison to the chow diet, the tracer decayed more rapidly with cholesterol feeding, resulting in smaller integrals for the function. The body cholesterol fractional catabolic rate increased threefold for the controls and doubled for the apoE-deficient homozygotes in comparison to chow feeding (**Table 3**). Body cholesterol transport nearly tripled in both homozygotes and normals. However, while the plasma cholesterol, R, and total
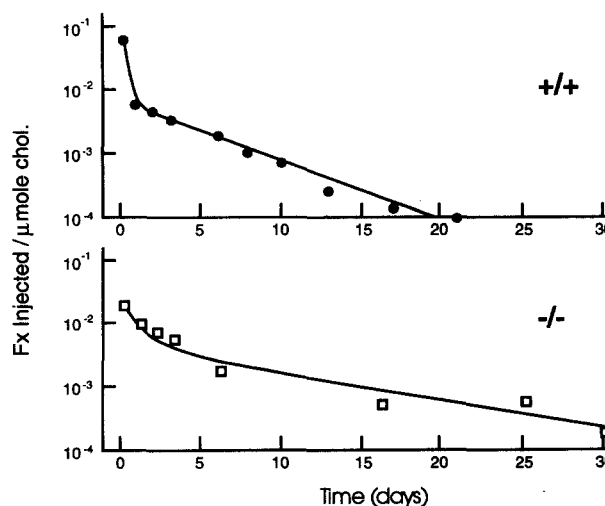

Fig. 2. Plasma cholesterol decay in cholesterol-fed normal and apoE-deficient mice. The data are representative plasma cholesterol specific activity functions after the intravenous injection of [1,2-³H]cholesterol in normals (+/+, top panel) and homozygotes (-/-, bottom panel) fed 1% cholesterol.

TABLE 3. In vivo cholesterol transport in cholesterol-fed normal and homozygote apoE-deficient mice

	Fractional Transfer Rates Day⁻¹			Pool Size μmol		Transport	
	R→S	S→R	FCR	R	Total	μmol/day	μmol/day/10 g
+/+, n = 6	1.56 ± 0.4	0.43 ± 0.1	1.58 ± 0.15 (1.1–2.3)	12 ± 1	59 ± 11 (39–105)	19.9 ± 3.4	8.4 ± 1.3
−/−, n = 4	1.70 ± 0.4	0.92 ± 0.2	0.33 ± 0.03[a] (0.26–0.43)	73 ± 15[b]	166 ± 21[b] (117–211)	22.5 ± 3.0	10.8 ± 1.0

Control mice and apoE-deficient mutants were maintained on 1% cholesterol, 10% lard diets for 4 weeks prior to study. Serum and tissue cholesterol contents are described in Table 2. Data are means ± SE of indicated mice number. Mean ± SE weights of mutants were 21 ± 2[g] and controls 24 ± 3[g]. The values within parentheses are in the ranges for these parameters.
[a] Significantly different than control at $P < 0.001$.
[b] Significantly different than control at $P < 0.01$.

body cholesterol were substantially unchanged in the controls, each of these parameters increased considerably in homozygotes. Most of the cholesterol in chow-fed mutant mice was in R, but this changed with cholesterol feeding. The slow turnover pool of the homozygotes now contained the most cholesterol. The appreciable rise in both the body cholesterol fractional catabolic rate and cholesterol transport of the homozygotes in response to cholesterol feeding indicates that the cholesterol transport of these mice is not saturated and can be increased. However, this increase in cholesterol transport is achieved only with ensuing increases in plasma and body cholesterol.

Cholesterol content in the liver of control animals doubled in response to a high cholesterol diet. This increase was primarily in hepatic cholesteryl ester. There was little change in other tissues of control mice. The opposite was true for the homozygous mutants. There was no change in the hepatic cholesterol content, particularly cholesteryl ester, while significant increases in peripheral tissue

cholesterol and a shift to cholesteryl ester were observed after a high cholesterol diet (Table 2). Studies stressing the system with dietary cholesterol emphasize the importance of apoE-dependent hepatic uptake in protecting plasma and peripheral tissues from elevations in plasma cholesterol concentrations when transport is increased.

Lovastatin treatment of two homozygous mice resulted in a significant and paradoxical increase in plasma cholesterol concentration for both; 10.7 ± .5 and 12.6 ± 1.2 μmol/ml prior to and 18.7 ± 1.6 and 20.9 ± 1.7 μmol/ml, respectively, during treatment (n = 5 for each, $P < 0.001$ and $P < 0.01$, respectively). No significant change was noted in the plasma cholesterol concentrations of control mice on lovastatin; 2.5 ± .1 prior to (n = 5) and 2.3 ± .1 μmol/ml (n = 6) during treatment. Plasma tracer cholesterol decay in the homozygotes was more rapid on the lovastatin (Fig. 3) than on chow (Fig. 1). Body cholesterol fractional catabolic rate almost tripled and body cholesterol transport doubled on treatment with the inhibitor of cholesterol synthesis (Table 4). Similar to the expanded body cholesterol with cholesterol feeding, the increased transport of endogenous cholesterol also resulted in higher body cholesterol contents. Also analogous to cholesterol feeding, most of the mass increment was found in the slow turnover pool. Control mice responded to lovastatin treatment with no change in plasma cholesterol, a modest increment in body cholesterol fractional catabolic rate, and decreased pool R and body cholesterol mass when compared to unsupplemented chow.

To evaluate the relevance of mutant plasma lipoprotein cholesterol transport to the observed changes in body cholesterol kinetics, cholesterol-labeled lipoproteins in the serum of mutants were injected into chow-fed controls and homozygotes. Homozygote plasma lipoprotein cholesterol clearance was considerably delayed in mutants compared to normals (Fig. 4). When these data were analyzed in a one-compartment system, the fractional mutant lipoprotein cholesterol clearance rate was 0.6 ± 0.02/h in normals and 0.07 ± 0.004/h in homozygotes. For the homozygote this would result in a plasma cholesterol transport that was two to three times that of total body
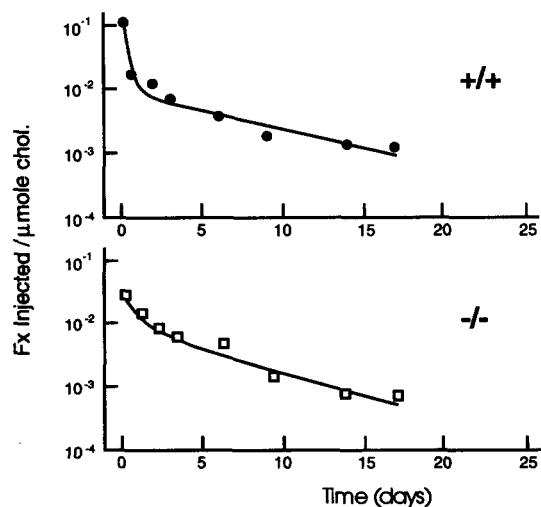

Fig. 3. Plasma cholesterol decay in lovastatin treated normal (+/+) and apoE-deficient (−/−) mice. The data are representative plasma cholesterol specific activity functions after the intravenous injection of [1,2-³H]cholesterol in normals (top panel) and homozygotes (bottom panel) fed 0.025% lovastatin.

TABLE 4. In vivo cholesterol transport in chow-fed, lovastatin-treated normal and homozygote apoE-deficient mice

	Fractional Transfer Rates Day^{-1}			Pool Size μmol		
	R→S	S→R	FCR	R	Total	Transport μmol/day
+/+, n = 2	0.91 ± 0.02	0.31 ± 0.04	0.77 ± 0.04	9 ± 1	32 ± 4	6.8 ± 0.9
−/−, n = 2	0.42 ± 0.04	0.31 ± 0.04	0.47 ± 0.04	35 ± 2	87 ± 12	16.5 ± 0.8

Control mice (mean ± SD wt 23.5 ± 2 g, n = 2) and homozygous apoE-deficient (mean wt 26.6 ± 3.0 g, n = 2) were fed 0.025% lovastatin in standard rodent chow for 4 weeks prior to study. Data are the means ± SD of duplicates of mutants and controls.

catabolic transport. Similar estimates cannot be made for the normals because total plasma cholesterol transport was not measured. Transfer of mutant lipoprotein cholesterol to other pools, i.e., high density plasma lipoproteins and biliary sterols, was much less for homozygotes than normals (**Fig. 5**). Normals demonstrated return of injected cholesterol to plasma high density lipoproteins, mainly as free cholesterol (Fig. 5A). This was similar to that observed for rat chylomicron cholesterol (19) and occurred at a time described (20) for hepatic chylomicron cholesteryl ester hydrolysis.

Fig. 4. Lower density plasma lipoprotein cholesterol tracer decay. The data are the mean ± SE d < 1.063 g/ml lipoprotein cholesterol specific activities of four homozygotes (top panel) and three normals (lower panel) after the injection of homozygote cholesterol-labeled lipoproteins (> 65% cholesteryl ester).

Sequential specific activities in bile acids of normal and homozygous mice were significantly different after injections of mutant lipoproteins (Fig. 5B). Specific activity in homozygotes was one-fifth the normal and did not demonstrate the progressive increase as did controls. The bile acid pool sizes obtained from the dilution of [^{14}C]taurocholate were 6.0 ± 1.5 μmoles (n = 5) for normals and 3.5 ± 0.03 μmoles (n = 6) for homozygotes. The lower specific activities of the mutant bile acids could not be explained by considerably larger bile acid pools with dilution in the mutant. Biliary neutral sterol specific activities were also about five-fold greater for normals than homozygotes (Fig. 5C). For both types of mice, the neutral sterol specific activities were considerably higher than the bile acid after receiving the cholesterol-labeled lipoproteins from the mutants.
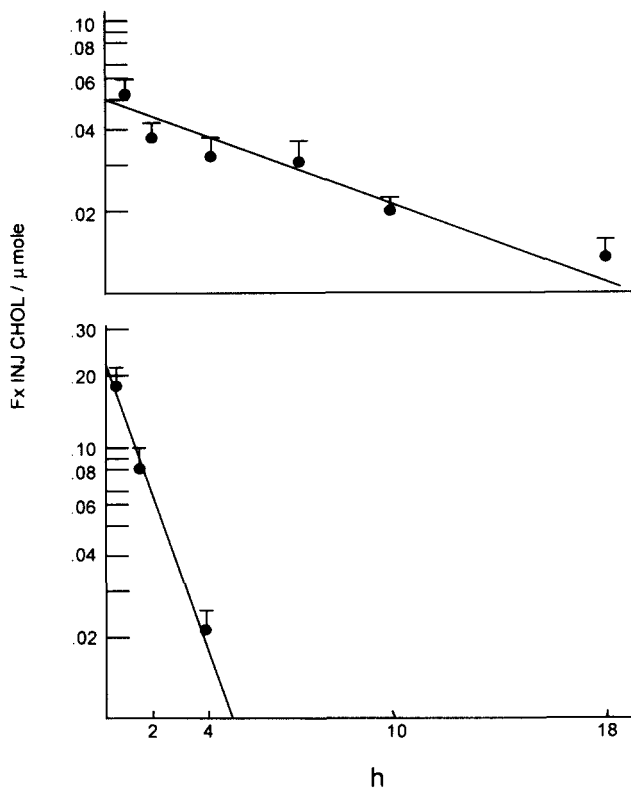
DISCUSSION

The absence of apoE results in a considerable increase of plasma cholesterol in both humans (21) and mice (6). Plasma lipoproteins accumulating in such mutations have physical characteristics and increased apoB-48 content similar to chylomicron and very low density lipoprotein remnants. These lipoproteins would be anticipated (1) to be poorly cleared without apoE. They differ, however, from the remnants of hepatectomized rats (22) and humans with Type III (23), having appreciably less triglyceride than either. Possibly, apoE-independent lipoprotein transport results in longer plasma residence times with greater exposures to lipase activity.

Impaired body cholesterol catabolism is consistently observed in homozygotes with apoE-independent plasma lipoprotein transport. Compared with controls, this defect is characterized by lower body cholesterol fractional catabolic rates and an increased body cholesterol mass for each mutant evaluated. The mice were studied during differing rates of transport and with differing sources of cholesterol entering the body. Irrespective of these conditions, the mutants always had a consistently larger body cholesterol mass, a greater distribution of this mass in the
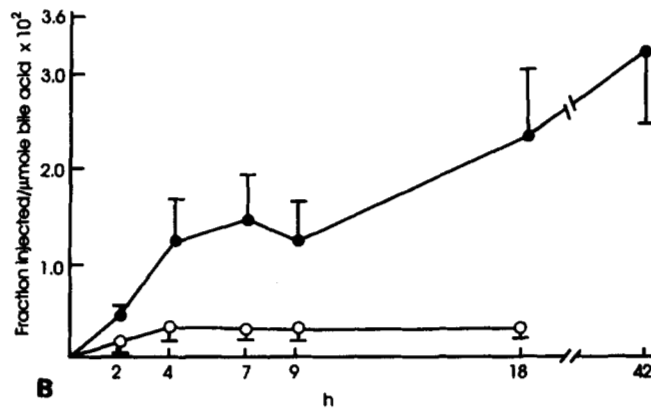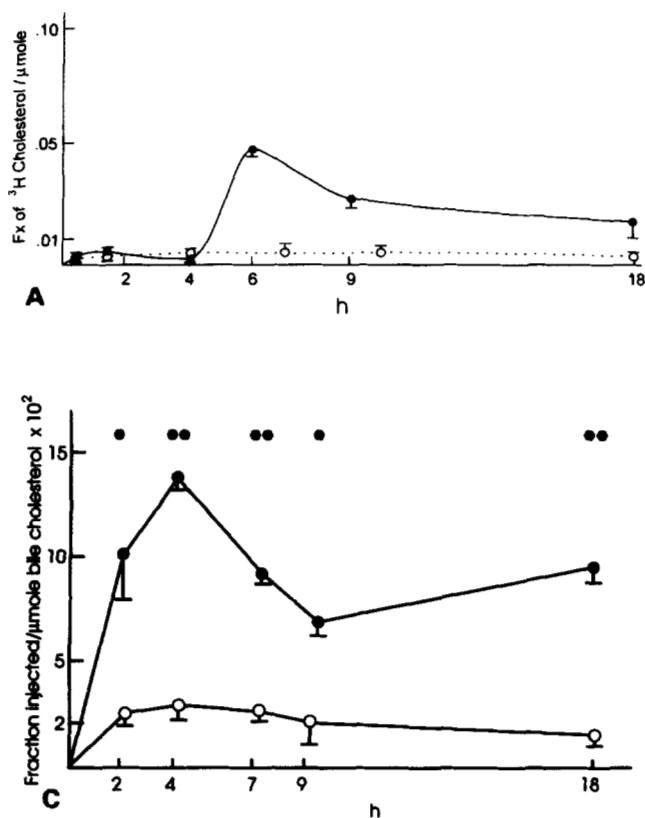
Fig. 5. The appearance of homozygote plasma lipoprotein cholesterol in other pools of normals and apoE-deficient mice after the injection of homozygous cholesterol-labeled lipoproteins. A: The data are the mean ± SE d > 1.063 g/ml lipoprotein cholesterol specific activities of four homozygotes (O) and three normals (●). The percent free cholesterol was 65 ± 5 for the normals at 6 h and 39 ± 4 for homozygotes at 7 h. B: Sequential bile acid specific activities after the injections of cholesterol-labeled homozygous lipoproteins. The normals values (●) are the means ± SE of four studies and the homozygote (O) data are the means ± SE of five animals. By repeated measures analysis of variance (18) homozygotes were significantly different than controls at $P < 0.015$. C: Sequential biliary neutral sterol specific activity corresponding to the data of Fig. 5B. The time intervals with a single asterisk are times when the normal and homozygote data are significantly different at $P < 0.01$ and the double asterisk, at $P < 0.001$.

rapid turnover pool, and lower body cholesterol fractional catabolic rates than respective controls.

Despite the catabolic impairment, homozygous apoE-deficient mice were able to transport as much cholesterol as controls during standard chow feeding. It is important to note, however, that this occurred at plasma cholesterol concentrations five times the control and with significant expansions of total body cholesterol, which is totally due to accumulation in R. The similarity of mutant and control tissue cholesterol contents after chow feeding indicate that R is primarily extracellular and at least half of this increment can be ascribed to plasma lipoproteins. These observations, along with the markedly delayed plasma lipoprotein cholesterol clearance in the apoE-deficient mouse, puts the catabolic defect at the level of cell cholesterol uptake. The reduction in tracer cholesterol appearance in biliary sterols and high density lipoprotein free cholesterol, after plasma lipoprotein cholesterol clearance, demonstrates the involvement of the liver in this defect. Even though the fractional transfer of plasma lipoprotein to biliary sterols is less for the mutants, the transport of mass is similar to control. This is consistent with a low affinity process, which produces an expanded pool size to maintain the same transport as occurs in a high affinity system without changes in pool size.

The similarity of control and mutant cholesterol inputs during chow feeding, where about 85% of this input is synthetic, is of interest. Endogenous cholesterol production in chow-fed mutants is not up-regulated. This may mean that apoE-independent transfer into cells, primarily hepatocytes, down-regulates synthesis as efficiently as does the transfer dependent on this apolipoprotein. The fact that similar amounts of cholesterol transit the liver in mutants and controls may be more important than the route of entry. The similarity in synthesis may also mean that regulation is more importantly influenced by another apolipoprotein (i.e., B-100) than apoE in the chow-fed state.

In the lovastatin-treated mutant, synthesis was up-regulated in contrast to controls. This treatment of normal rats results in an increase in the mass of 3-hydroxy-3-methylglutaryl-CoA reductase, and a doubling of cholesterol synthesis within 6 h after the last dose of lovastatin (24). The difference between control and apoE-deficient mutants indicates an important role of apoE-mediated cholesterol transfer in suppressing the synthetic increase that can occur between doses of the inhibitor. This observation and the decrease in total body cholesterol fractional catabolic rate suggest that apoE importantly affects plasma cholesterol access to liver, for both catabolism and synthetic regulation. The paradoxical rise in plasma cholesterol that occurred with lovastatin suggests either that the up-regulation of LDL receptors

produced by this agent had not occurred in these mutants or that apoE-independent plasma clearance was not mediated by this receptor. These issues are under study.

Lovastatin and cholesterol feeding enhanced endogenous and exogenous body cholesterol transport, respectively, in the apoE-deficient mutant. The large expansion of plasma and body cholesterol needed during chow feeding to produce mutant transports similar to normals could suggest that this was maximum transport in a saturated system. The results with lovastatin and cholesterol supplementation demonstrate considerable capacity to transport body cholesterol from synthetic and exogenous sources. The enhanced body cholesterol transport during cholesterol feeding resulted not only from an increase in the catabolic (R) pool size, but a doubling of the body cholesterol fractional catabolic rate as compared to chow. Lovastatin treatment produced a tripling of fractional catabolic rate in the mutant with little change in R. The enhanced catabolic efficiency (i.e., fractional catabolic rate) demonstrated by mutants when stimulated to transport more cholesterol possibly relates to changes observed in plasma lipoproteins. If plasma lipoprotein cholesterol access to liver importantly influences body cholesterol FCR, the higher concentrations of these lipoproteins during cholesterol or lovastatin feeding should facilitate greater cellular access by a low affinity transport system. Secondly, a potential shift in size to larger lipoproteins should result in greater cholesterol entry into cells with each lipoprotein particle. This adaptive catabolic response by the mutants is under investigation.

Cholesterol feeding increased body cholesterol transport to a similar extent in both controls and homozygotes indicating that intestinal cholesterol absorption was not attenuated in the absence of apoE. The normal mouse responds to this enhanced body cholesterol transport with a modest hepatic cholesterol increase and no change in other tissues including plasma. In the absence of apoE, this is reversed so that the liver revealed little change in cholesterol, while peripheral tissues, particularly plasma, demonstrated considerable increments. These data and those from lovastatin feeding demonstrate the importance of the high affinity apoE-mediated hepatic lipoprotein access pathway in the maintenance of normal plasma and peripheral tissue cholesterol concentrations when transport is increased. In the normal mouse, an increase in hepatic rather than plasma cholesterol occurs, with greater transport indicating that apoE-mediated plasma lipoprotein access to liver saturates later than other hepatic cholesterol transfer processes. In the homozygote, where hepatic access appears to be a limiting step in body cholesterol transport, a much more impressive increase in plasma concentration occurs. The appreciable cholesterol accumulation in peripheral tissues quite likely results from this plasma increment. High affinity apoE-mediated cholesterol transfer does not require increased plasma

concentrations to increase body cholesterol transport. The subsequent peripheral tissue increments and their pathologic consequences are avoided.

. The absence of apoE in mice results in an increment of plasma lipoprotein cholesterol concentration. This cholesterol was primarily in lower density, remnant-like lipoproteins, rather than the high density lipoproteins of normals. This mutant plasma lipoprotein cholesterol increment resulted from defective plasma clearance in the chow-fed mouse and comprised at least half of the increase in total body cholesterol observed. This rise in body cholesterol mass and the appreciably lower body cholesterol fractional catabolic rate clearly demonstrate an abnormality of body cholesterol catabolism in the apoE-deficient mutant. It is possible that the plasma lipoprotein clearance defect is the primary, and even the sole, cause of this catabolic abnormality but at present this is uncertain. Despite the abnormal catabolism, the mutant was able to transport at least as much cholesterol as controls in each of the three metabolic conditions studied. Cholesterol transport capacity in mutants via the apoE-independent low affinity system was equivalent to normals, but always at considerable expansions of plasma and body cholesterol mass. ▦

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