Effects of dietary fat on cholesterol movement between tissues in CBA/*J* and C57BR/*cdJ* mice[1]

Son I. Kuan,[2] Jeanne Stewart, Michael K. Dowd,[3] Leah Patterson,[4] Jacqueline Dupont,[5] and Richard C. Seagrave[6]

Departments of Chemical Engineering and Food Science and Human Nutrition, Iowa State University, Ames, IA 50011

Abstract Differences in dietary fats cause differences in cholesterol metabolism in mice. CBA/*J* mice are resistant to diet-induced hypercholesterolemia and atherosclerosis; they adjust hepatic hydroxymethyl-glutaryl-CoA reductase activity (HMGR) to maintain homeostasis; C57BR/*cdJ* mice are susceptible, but young animals are thought to maintain homeostasis by changing fecal excretion of sterols. Compartmental modelling of movement of [4-^{14}C]cholesterol was used to analyze movement of cholesterol between serum and liver, heart, and carcass in mice fed 40 en% fat, polyunsaturated to saturated fatty acid ratio (P/S) = 0.24 (US74) or 30 en% fat, P/S = 1 (MOD). Dietary effects were quite pronounced, while strain effects were more subdued. The C57/*cdJ* animals appear to regulate the overall cholesterol balance by reducing synthesis, as do the CBA/*J* animals, even though synthesis is not reduced to the same degree as in the CBA/*J* animals. Both diet and strain influence the whole-animal turnover rate, with slower turnover occurring for C57BR/*cdJ* animals and animals fed the US74 diet.—**Kuan, S. I., J. Stewart, M. K. Dowd, L. Patterson, J. Dupont, and R. C. Seagrave.** Effects of dietary fat on cholesterol movement between tissues in CBA/*J* and C57BR/*cdJ* mice. *J. Lipid Res.* 1992. **33:** 1619–1628.

Supplementary key words cholesterol synthesis • saturated fat • polyunsaturated fat

The genetic regulation of plasma cholesterol concentration by lipoproteins has been quite thoroughly documented (1). Defective low density lipoprotein (LDL) receptors account for a large portion of the human hypercholesterolemias, but there are many genetic sites involved in LDL receptor function. Cholesterol balance is a result not only of LDL clearance regulated by the receptors, but also of absorption from the intestine, removal from peripheral cells, and excretion as neutral sterol or bile acids via the intestine (2). The response to dietary fat is different among individuals; some are especially susceptible to diet-induced hypercholesterolemia (high cholesterol, high saturated fat), whereas others are particularly resistant (3). The genetic regulation of diet effects on cholesterol metabolism is not understood, but diet constitutes a major aspect of therapy for hypercholesterolemia (4).

A useful model for the study of diet/genetic interaction is the inbred mouse. The CBA/*J* strain is hyporesponsive and the C57BR/*cdJ* strain is hyperresponsive to atherogenic diets in respect to development of atheromatous plaques after 14 weeks consumption of the Thomas-Hartroft type diet (5, 6). Others have reported hypercholesterolemia with such diets (7–9). We have shown that these strains do not develop hypercholesterolemia if the mice are fed a diet corresponding to the average fat composition of the American diet in 1974 (40 en% fat, polyunsaturated fatty acid to saturated fatty acid ratio (P/S) of 0.24, 47 mg cholesterol/1000 kcal, US74 diet) (10). Previous evidence indicates that the two strains maintained cholesterol homeostasis by different mechanisms. The CBA/*J* mouse adjusted hepatic hydroxymethyl-glutaryl coenzyme A reductase (HMGR) activity, and the C57BR/*cdJ* mouse changed fecal excretion of cholesterol (11).

Abbreviations: LDL, low density lipoproteins; en %, energy percent. Symbols used in text: E, mass flow rate of cholesterol leaving the system by excretion or by conversion to bile acids, mg/h; I, mass flow rate of ingested cholesterol, mg/h; M_i, mass of total cholesterol in the ith compartment, mg; R_{ij}, mass flow rate of total cholesterol from ith compartment to jth compartment, mg/h; Sy, mass flow rate of synthesized cholesterol, mg/h; X_i, specific activity of total cholesterol in the ith compartment, dpm/mg; X_D, dose remaining at injection site, dpm; τ, dose mean residence time, h. Subscripts: C, carcass compartment; D, dose compartment; H, heart compartment; L, liver compartment; S, serum compartment.

[1]Journal Paper No. J-14036 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project 2571, a contributing project to North Central Regional Research Project NC-167.

[2]Present address: Department of Medicine, Box 135, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

[3]Present address: Institut National de la Recherche Agronomique, Laboratoire de Physicochimie des Macromolecules, Rue de la Geraudiere, 44026 Nantes Cedex 03, France.

[4]Present address: Union Carbide Corporation, South Charleston, WV 25309.

[5]Present address: Agricultural Research Services, USDA, Beltsville, MD 20705.

[6]To whom correspondence should be addressed.

Plasma cholesterol concentration may not be a reflection of organ concentrations. The momentary plasma concentration is the result of complex interactions among organs, modified by dietary components, synthesis, and excretion (**Fig. 1**). To gain insight into the diet/genetic interaction on whole-body cholesterol metabolism kinetic aspects, we fed CBA/*J* and C57BR/*cdJ* mice the US74 diet and a modified diet containing the fat composition suggested by the Dietary Goals of the United States (12) as used in the earlier study (11). [4-^{14}C]cholesterol was used to trace movement of cholesterol between serum and liver, heart, and carcass. The data were analyzed by phenomenological compartmental modelling. The model allowed calculation of synthesis rates, excretion rates, and half-lives of the sterol nucleus.

In this work a dynamic model is developed with its structure based on the known physiology and biochemistry of the system. The model equations are solved numerically to directly yield the model parameters using a nonlinear, multiple response, regression procedure. A common problem with some recent cholesterol models is that some are more complex than justified by the type or accuracy of the data to which they are matched (13). One common technique is to study the dynamics of plasma cholesterol turnover and infer the dynamics within other sites in the body (e.g. ref. 14). If only one tissue is sampled, for example the plasma, it is difficult to predict accurately behavior within other tissues. Variance in the experimental data can make it difficult to discern which set of parameters to a model or which model gives the best fit when deconvolution is used. Also if only one tissue is sampled, it usually is not possible to properly account for the total amount of the dose given or to determine if it has actually been recovered during the course of the experiment.

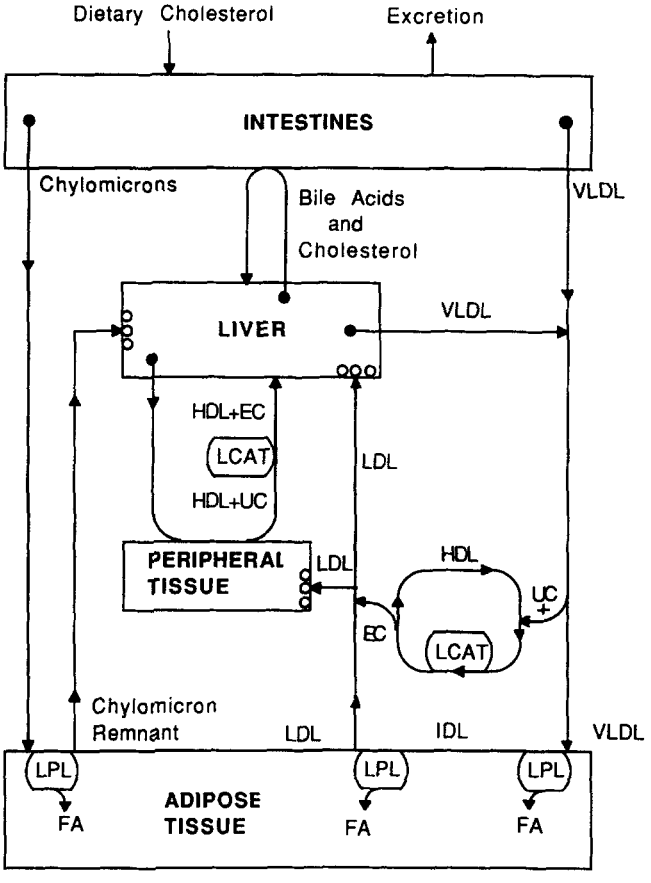

Fig. 1. Pathways for cholesterol movement; LPL, lipoprotein lipase; LCAT, lecithin:cholesterol acyltransferase; FA, free fatty acids; EC, esterified cholesterol; UC, unesterified cholesterol; ●, synthesis sites; ○, LDL receptors.

METHODS

Experimental design

C57BR/*cdJ* mice (susceptible to diet-induced atherosclerosis) and CBA/*J* mice (resistant) were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred in the Animal Care Facility of the Food and Nutrition Department, Iowa State University. Breeders and post-weaning offspring were fed a commercial diet (Purina Breeder Chow, St. Louis, MO). Six-week-old males were placed on experimental diets. The diets were the same as described previously (11). The US74 diet was formulated to contain fat components similar to the average diet in the United States according to the HANES I survey (12): 40 en% fat with a P/S of 0.3, 300 mg cholesterol/1000 kcal. The modified diet (MOD) was designed to meet the Dietary Goals for the United States (12): 30 en% fat with 10 en% saturated, 10 en% monounsaturated, and 10 en% polyunsaturated fatty acids, <150 mg cholesterol/1,000 kcal. The purified diets contained isoenergetic concentrations of protein, vitamins, and minerals, and only differed in carbohydrate and fat (11). The mice were fed ad libitum and were caged singly in a room with controlled temperature (72°C) and humidity (50%). After 8 weeks consuming the experimental diets, each mouse was injected intraperitoneally with 1.1 × 10^6 dpm [4-^{14}C]cholesterol in an ethanol-saline suspension. Two series of studies were performed; in the first, mice were killed at 1, 2, 4, 6, 10, 13, 16, 20, 23, 26, 30, and 34 days after injection (long-term study). Because the first samples were taken 24 h after injection, the peaks of some of the specific activity curves were not observed. In the second series, mice were killed at 0.5, 1, 2, 4, 6, 12, 18 h and 1, 2, 3, 4, 5, 6, and 7 days post-injection (short-term study). The total number of mice was 380, and 3–8 mice were used per time point. An experiment was conducted to determine whether fasting versus feeding affected the specific activity of the tissue cholesterol; no effect was found.

Tissue analyses

At the appropriate time, mice were anesthetized with ether and killed by decapitation. Blood was collected and allowed to clot at room temperature. Serum was prepared by centrifuging the blood at 2,000 *g* for 10 min (Beckman Model J-6B centrifuge) and stored at −80°C until analysis. The liver, heart, and eviscerated carcass were frozen and stored in the same way. The head was not included in the carcass samples because of the slow exchange rate of cholesterol with the brain. In this first model, the kidneys, lungs, bladder, and testes also were neglected. Avigan, Steinberg, and Berman (15) have shown that the specific activity of labeled cholesterol in the kidneys and lung tissue of rats can be of the same order of magnitude as that in the liver tissue. Although these organs may be exchanging cholesterol, their cholesterol masses are all small relative to the carcass and liver, and, therefore, they will account for only a small portion of the labeled cholesterol. Hence, the exchange between these organs can be neglected without greatly affecting the results for the compartments considered. Because of the complication of unabsorbed dietary cholesterol, the gastrointestinal tract was also excluded.

The samples were saponified in 10% KOH in 50% ethanol for differing lengths of time. The nonsaponifiable material was extracted with Skelly B petroleum ether (n-hexane). Problems encountered during the first experimental series were corrected in the second series. Incomplete extraction of the cholesterol during the initial long-term study resulted in low estimates of the component cholesterol masses. In series 2 (0.5 h to 7 days) the hearts and livers were extracted by the procedure of Folch Lees, and Sloane Stanley (16) before saponification. Only the short-term data were used in estimating these masses. In addition, the saponification time (4 h, 80°C) and number of extractions (two) of the first series were not sufficient for complete extraction of the carcass. The problem was corrected in the second series (48 h, 75°C, four extractions). By using data from overlapping points of the two series (1, 2, 4, and 6 days), a correction factor was calculated and applied to the carcass data from the first series. The petroleum ether extract was used to determine cholesterol mass and radioactivity (17). The scintillation fluid, Scintilene (Fisher Scientific Co.), was used to dissolve the sample, and radioactivity was determined by using a Packard TRI-CARB liquid scintillation spectrometer. Corrections were made for quenching and the data were converted to dpm. Specific activities of cholesterol of serum, heart, liver, and carcass were calculated, and all data were expressed in relation to the same dose of radioactivity and normalization to a 50-g mouse.

Model development

A simple model of whole-body cholesterol movement has been developed that is consistent with known anatomical structures and physiological functions. The basic model is chosen to have four compartments, one for each of the four tissues (serum, liver, heart, and carcass) that were sampled experimentally. The four compartments are assumed to be an appropriate approximation to a whole animal and to correspond with what could be sampled experimentally. Each compartment is considered to be a perfectly mixed pool of total (free and esterified) cholesterol. Hellman et al. (18) have shown experimentally that soon after administration of labeled cholesterol, labeled free and esterified cholesterol seem to be in equilibrium and, therefore, can be treated as indistinguishable. Unlabeled total cholesterol movement in the animal was considered to be at steady state. Previous results have shown that this is appropriate when an adult animal has been fed an experimental diet for 8 weeks and has been injected with a relatively small amount of labeled cholesterol. By using the steady-state hypothesis, four independent material balances can be written for total cholesterol, one for each compartment. It should be noted that the combination of a model with well-mixed compartments and the steady-state assumption will conceptually exclude 'active transport'-like mechanisms of the label between compartments (i.e., the label cannot be accumulated against its concentration gradient).

As mentioned above, labeled cholesterol was considered to be treated identically to unlabeled cholesterol by the body. For this assumption to hold, the basic model had to be modified to properly treat the observed behavior of the dose of label. Nilsson and Zilversmit (19) have shown that when labeled cholesterol in an ethanol–saline suspension is injected intravenously, it is rapidly phagocytized by the Kupffer cells that line the blood sinusoids in the liver. Within 24 h, the labeled cholesterol is released back into the plasma, primarily in unesterified form. In the present study, [4-^{14}C]cholesterol in an ethanol–saline suspension was injected intraperitoneally into the capillary-rich fat pads that line the abdomen. It is proposed that in this instance a similar process occurs whereby the dose enters the capillaries or lymphatic ducts, is phagocytized by the Kupffer cells, and then released back into the plasma. Because this route is only available for the transport of labeled cholesterol, an additional dose compartment and nonphysiological pathway from the injection site (dose compartment) to the Kupffer cells (liver compartment) was added to the model. The label was considered to leave the dose compartment (a subdivision of the carcass compartment) by simple wash-out. Once the labeled choles-

terol enters the liver it is assumed to be indistinguishable from the existing unlabeled cholesterol. The labeled cholesterol in the dose compartment was considered unable to enter the system by diffusion into the carcass compartment. Experimental considerations leading to the addition of this compartment will be discussed later.

Cholesterol is assumed to leave the system from the liver compartment by excretion in the neutral form or by conversion to bile acids. Only two physiological pathways were considered for cholesterol to enter the system. First, ingested cholesterol, after being absorbed via the small intestine, was assumed to be released into the plasma compartment; second, cholesterol could be synthesized in the liver compartment. The cholesterol ingestion rate was calculated from the known composition of the diets and the measured food intake. Both values were determined during the experiments and values have been reported previously (11). These rates were taken as fixed constants equal to the dietary input of cholesterol after scaling to account for variations in body mass. The complete schematic of the model is shown in **Fig. 2.** All symbols are defined in the abbreviations.

For modelling purposes the dose and carcass compartments are summed to give a net carcass compartment, as only the net specific activity of label can be determined experimentally. Between the eight remaining flows in the steady-state balances, four independent equations can be written. These equations reduce the number of unknowns in the model leaving four variable cholesterol flows to be independently determined. We have chosen the excretion rate (E) and one of each of the pairs of internal exchange rates as the quantities determined by regression. With the dose compartment time constant this gives five unknown variables: R_{CS}, R_{HS}, R_{LS}, E, and τ.

For each compartment, the average of the specific activity at each time point was used in the model solution. The four model equations were solved simultaneously for the unknown parameters (R_{CS}, R_{HS}, R_{LS}, E, τ) using a multi-response minimization technique (20, 21). The objective function minimized is the determinant of the matrix formed from the product of the individual response errors (tissue errors). The main advantages of this technique are that there are no assumptions made about correlation of errors between tissues and the estimated standard errors, although of unknown accuracy, tend to

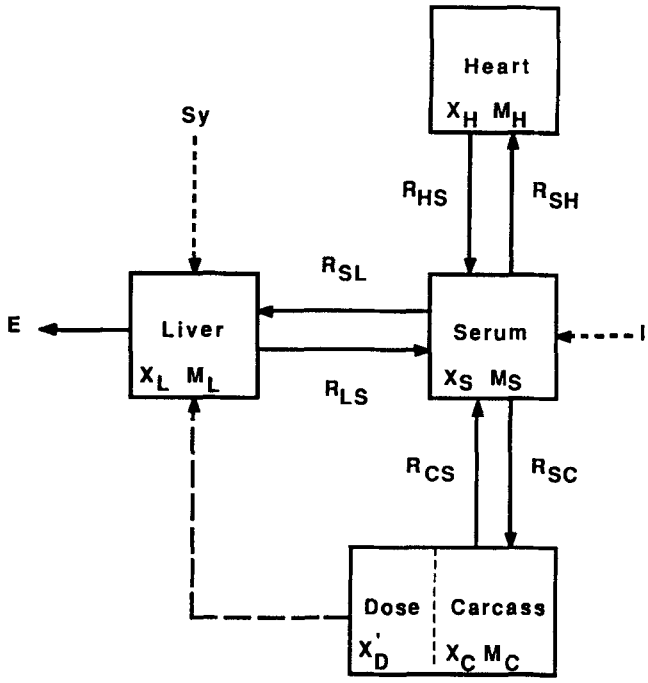

Fig. 2. Proposed compartmental model for whole-body cholesterol movement; (—) pathways available for both labeled and unlabeled cholesterol; (----) pathway available only to unlabeled cholesterol; (— — —) pathway available only to labeled cholesterol.

TABLE 1. Live-animal and whole-organ weights of CBA/*J* and C57BR/*cdJ* mice fed two different diets

Strain	Diet[a]	n	Live Wt[b]	Carcass[b]	Heart[b]	Liver[b,c]	Serum Total[b]
				g			*μl*
CBA/*J*	US74	56	44.34 (2.92)	31.82 (1.50)	0.174 (0.022)	2.85 (0.45)	339.6 (80.8)
CBA/*J*	MOD	55	43.52 (2.52)	31.65 (1.33)	0.173 (0.022)	2.04 (0.37)	340.6 (76.4)
C57BR/*cdJ*	US74	59	39.62 (6.22)	28.05 (4.76)	0.156 (0.015)	1.58 (0.38)	219.0 (69.9)
C57BR/*cdJ*	MOD	56	37.33 (5.54)	26.16 (4.42)	0.157 (0.022)	1.33 (0.22)	207.8 (65.1)

Values given as means; standard deviations in parentheses.
[a]Diets: US74: 40 en% fat, P/S = 0.3, cholesterol = 300 mg/1,000 kcal. MOD: 30 en% fat, P/S = 0.9, cholesterol = 47 mg/1,000 kcal.
[b]Generalized Linear Model (GLM) indicated significant strain effect, $P < 0.001$.
[c]GLM indicated significant diet effect, $P < 0.001$.

be smaller than those produced by uni-response techniques. The liver excretion rate (see Fig. 2) was integrated over time to estimate the depletion of the dose and to calculate a whole-animal cholesterol half-life.

The objective function was minimized by programming a function minimization procedure around a differential-equation integrator. A Numerical Algorithms Group (NAG) library subroutine E04JBF, based on a Quasi-Newton method, was used to perform the minimization. The initial value integrations were calculated with the International Math and Science Libraries (IMSL) route DIVPAG, based on the Gear method. Several additional NAG library routines were also required: F03AAF, to calculate the objective function determinant; F04AEF, to invert the estimate of the Hessian matrix; and D01GAF, for numerical integrations. Initial estimates of the regressed parameters were required, and a thorough search of the parameter space was needed to ensure locating the lowest minimum.

RESULTS

Organ weights and cholesterol concentrations

Actual weights of mice and organs from the 7-day experiment are shown in **Table 1.** A significant strain effect was found for each tissue mass ($P < 0.0001$). Diet appears to have a significant effect on the liver mass ($P < 0.0001$), but not on the other measured masses. **Table 2** lists averaged cholesterol tissue masses based upon normalization to a 50-g mouse. Data were analyzed by regression for time, and no statistical change over time was found within either series, supporting the use of the

TABLE 2. Organ cholesterol masses of CBA/*J* and C57BR/*cdJ* mice fed two different diets (scaled to a 50-g mouse)

Strain	Diet[a]	Serum[b]	Liver[b,c]	Heart[b]	Carcass[b]
			mg		
CBA/*J*	US74	0.78	13.78	0.26	90.8
		(0.19)	(4.37)	(0.05)	(12.9)
CBA/*J*	MOD	0.77	6.66	0.26	82.5
		(0.20)	(1.70)	(0.04)	(15.0)
C57BR/*cdJ*	US74	0.61	16.15	0.30	39.7
		(0.18)	(1.50)	(0.04)	(13.2)
C57BR/*cdJ*	MOD	0.57	12.00	0.31	46.8
		(0.15)	(1.36)	(0.05)	(22.1)

Values given as means; standard deviations in parentheses.
[a]Diets: US47: 40 en% fat, P/S = 0.3, cholesterol = 300 mg/1,000 kcal; MOD: 30 en% fat, P/S = 0.9, cholesterol = 47 mg/1,000 kcal.
[b]Generalized Linear Model (GLM) indicated significant strain effect, $P < 0.001$.
[c]GLM indicated significant diet effect, $P < 0.001$.

TABLE 3. Predicted cholesterol exchange flows from a whole-animal phenomenological model

	CBA/*J*		C57BR/*cdJ*	
Parameter	US74 Diet	MOD Diet	US74 Diet	MOD Diet
Regressed				
R_{CS} [= R_{SC}] (mg/h)	0.478[a,b]	0.203[a,b]	0.157[a,b]	0.274[a,b]
	(0.092)	(0.033)	(0.023)	(0.068)
R_{HS} [= R_{HS}] (mg/h)	0.010[a,b]	0.016[a,b]	0.030[a,b]	0.016[a]
	(0.001)	(0.001)	(0.003)	(0.002)
R_{LS} (mg/h)	0.832[a,b]	2.26[a]	1.301[b]	
	(0.180)	(0.58)	(0.201)	
E (mg/h)	0.387[a]	0.258[a,b]	0.386[a]	0.242[a,b]
	(0.031)	(0.015)	(0.018)	(0.022)
τ (h)	80.4[a,b]	141.3[a,b]	166.4[a,b]	153.9[a,b]
	(4.1)	(8.3)	(1.9)	(21.2)
Literature				
I (mg/h)	0.224[a,b]	0.019[a,b]	0.202[a,b]	0.016[a,b]
	(0.048)	(0.001)	(0.020)	(0.002)
Calculated				
R_{SL} (mg/h)	1.056[a,b]	2.279[a]	1.503[b]	
	(0.186)	(0.580)	(0.202)	
Sy (mg/h)	0.163[a]	0.239[a,b]	0.184[a]	0.226[a,b]
	(0.057)	(0.015)	(0.027)	(0.022)
Sy/E (%)	42.1[a]	92.6[a]	47.7[a]	93.4[a]
	(13.3)	(7.9)	(7.3)	(12.4)
Total excreted label after 34 days (% of dose)	90	81	93	88
Whole animal half-life (h)	146	234	203	279
	(6.1 days)	(9.7 days)	(8.5 days)	(11.6 days)

Estimated standard deviations and errors are in parentheses.
[a]Indicates a diet effect (*t*-test, n = 83, $P < 0.001$).
[b]Indicates a strain effect (*t*-test, n = 83, $P < 0.001$).

steady-state hypothesis (see above). Total serum cholesterol was based on serum volume measurements made after centrifugation and serum fraction collection. The CBA/*J* mice had more total serum cholesterol ($P < 0.0001$) than the C57BR/*cdJ* mice, and there was no significant effect of diet in either strain. Liver cholesterol concentration was affected by strain ($P < 0.0001$) and diet ($P < 0.0001$); CBA/*J* mice had less than the C57BR/*cdJ* mice, and those fed the US74 diet had more than those fed the MOD diet. A diet/strain interaction ($P < 0.0001$) suggests that the CBA/*J* mice had a relatively greater response to diet than did the C57BR/*cdJ* strain. The hearts of the C57BR/*cdJ* mice had more total cholesterol than those of the CBA/*J* strain ($P < 0.0001$), and diet had no effect. The CBA/*J* mice had greater carcass total cholesterol than did the C57BR/*cdJ* strain ($P < 0.0001$), and diet did not affect that value in either strain.

Rates of movement of cholesterol

Rates of cholesterol movement between the tissues considered in the model are shown in **Table 3** with the estimated standard errors and t-test comparisons. The model dynamic responses are shown plotted with the experimental data in **Figs. 3–6**. Because of the unknown accuracy associated with estimating standard errors from multiresponse regression techniques (21), inferences based on these values may also be inaccurate. In CBA/*J* mice the rates of movement between serum and liver were much greater than the rates between serum and heart or serum and carcass. Diet had an apparent effect upon movement between serum and liver, the rate for the MOD diet being almost three times the rate for the US74 diet ($P < 0.001$). A strain effect was found for this exchange flow for mice fed the US74 diet ($P < 0.001$). No value could be confidently determined for the liver to serum exchange for the C57BR/*cdJ* mice fed the MOD diet. Starting the optimization procedure from several different parameter values, the model would converge to the same values for all the other variables except this exchange flow, which often converged to different and very high values. Hence, no single value is reported (see Discussion). The rate of exchange between the carcass and serum was higher for the US74 diet in the CBA/*J* mice ($P < 0.001$) but lower for the C57BR/*cdJ* mice ($P < 0.001$). The opposite trends were found for the heart to serum exchange rates

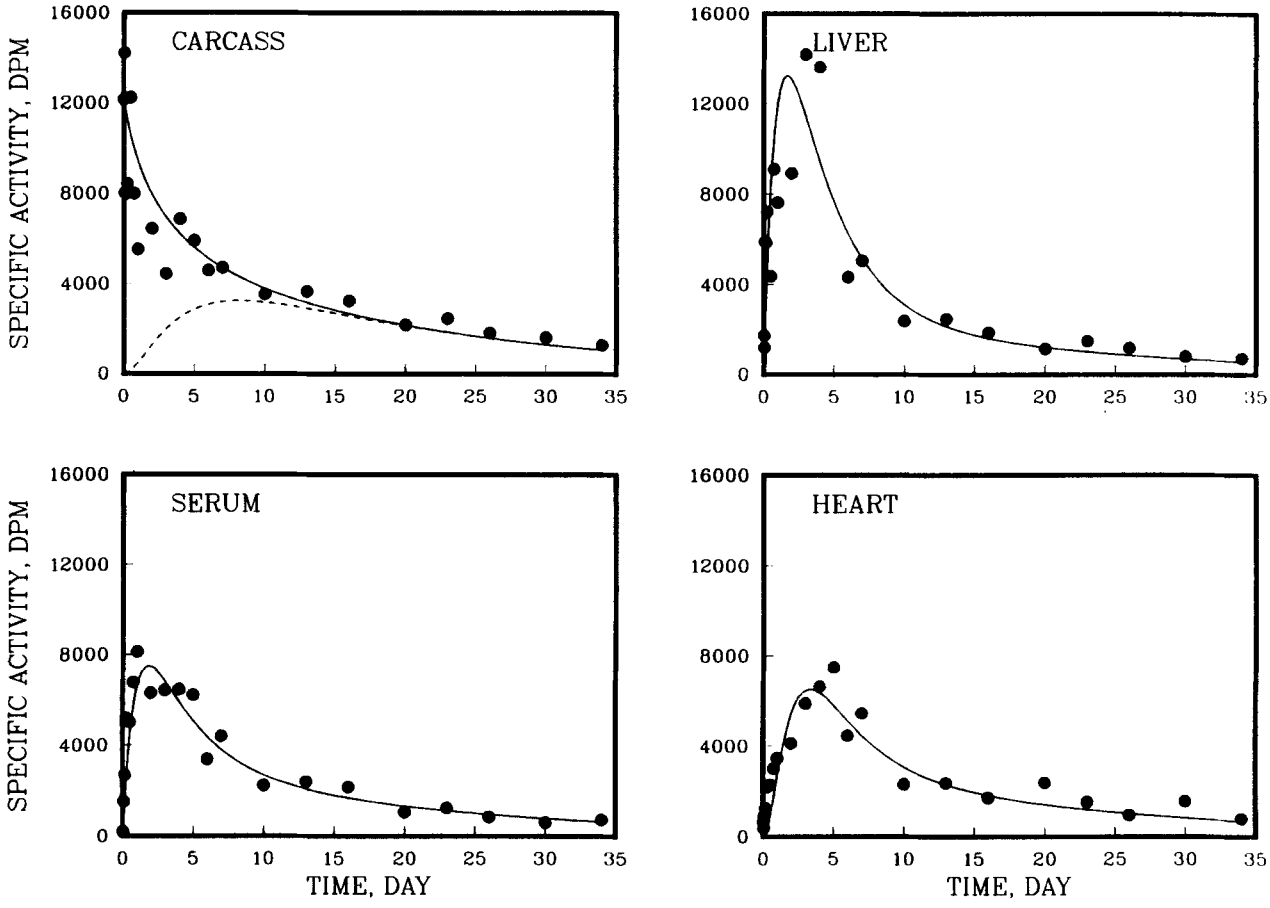

Fig. 3. Model and experimental labeled cholesterol versus time for CBA/*J* mice fed the US74 diet.

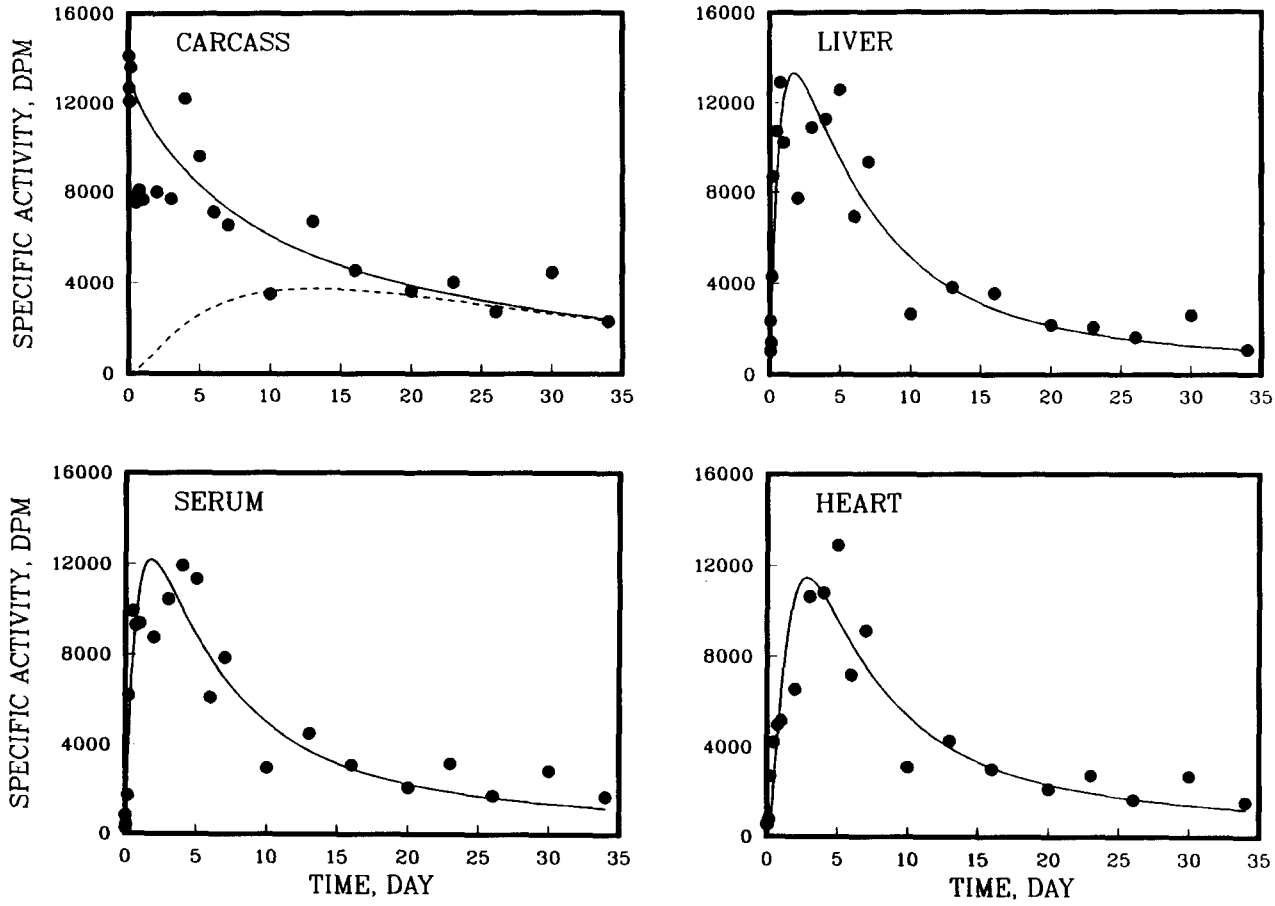

Fig. 4. Model and experimental labeled cholesterol versus time for CBA/*J* mice fed the MOD diet.

($P < 0.001$). The rate of excretion was greater for animals fed the US74 diet than for those fed the MOD diet ($P < 0.001$). Conversely, mice fed the US74 diet had a lesser rate of cholesterol synthesis than those fed the MOD diet ($P < 0.001$). The synthesis to excretion ratio reflects this result with the ratio being much greater for the mice fed the MOD diet.

As was seen experimentally, the model predicted that most of the labeled cholesterol had left the animals after the 34 days of the experiment. Whole-animal half-lives were longer for the C57BR/*cdJ* animals, and the animals fed the low-fat diets.

DISCUSSION

The mathematics of the model places certain restrictions on the type of dynamic responses that can be expected. Net exchange between two compartments must be proportional to the concentration difference within those two compartments because of the steady-state hypothesis. In addition, by treating the dose as passing directly from the injection site to the liver, the concentration of the label in the liver compartment must at any time be greater than that in the serum compartment. Conversely, if the dose was passed to the serum, the concentration in the liver could never be greater than in the serum. The observation by Nilsson and Zilversmit (17) that the label was rapidly sequestered by the liver and our observation that higher concentration peaks were found in the liver have led to the use of the former approach in the modelling. In general, this approach has been successful. The single difficulty we found was with the C57BR/*cdJ* mice fed the MOD diet where the experimental data are such that at some times the serum concentrations appear to be greater than in the liver. In this instance the model has difficulty mimicking the serum and liver data and hence predicting the serum to liver exchange since mathematics restricts the liver concentration to be greater than the serum concentration. In result the serum and liver exchange flows become very large and the modelled dynamic profiles become identical. It is not clear as to the physiological reason for this difference for the C57BR/*cdJ* experimental data, but it suggests that either the normally rapid initial uptake of

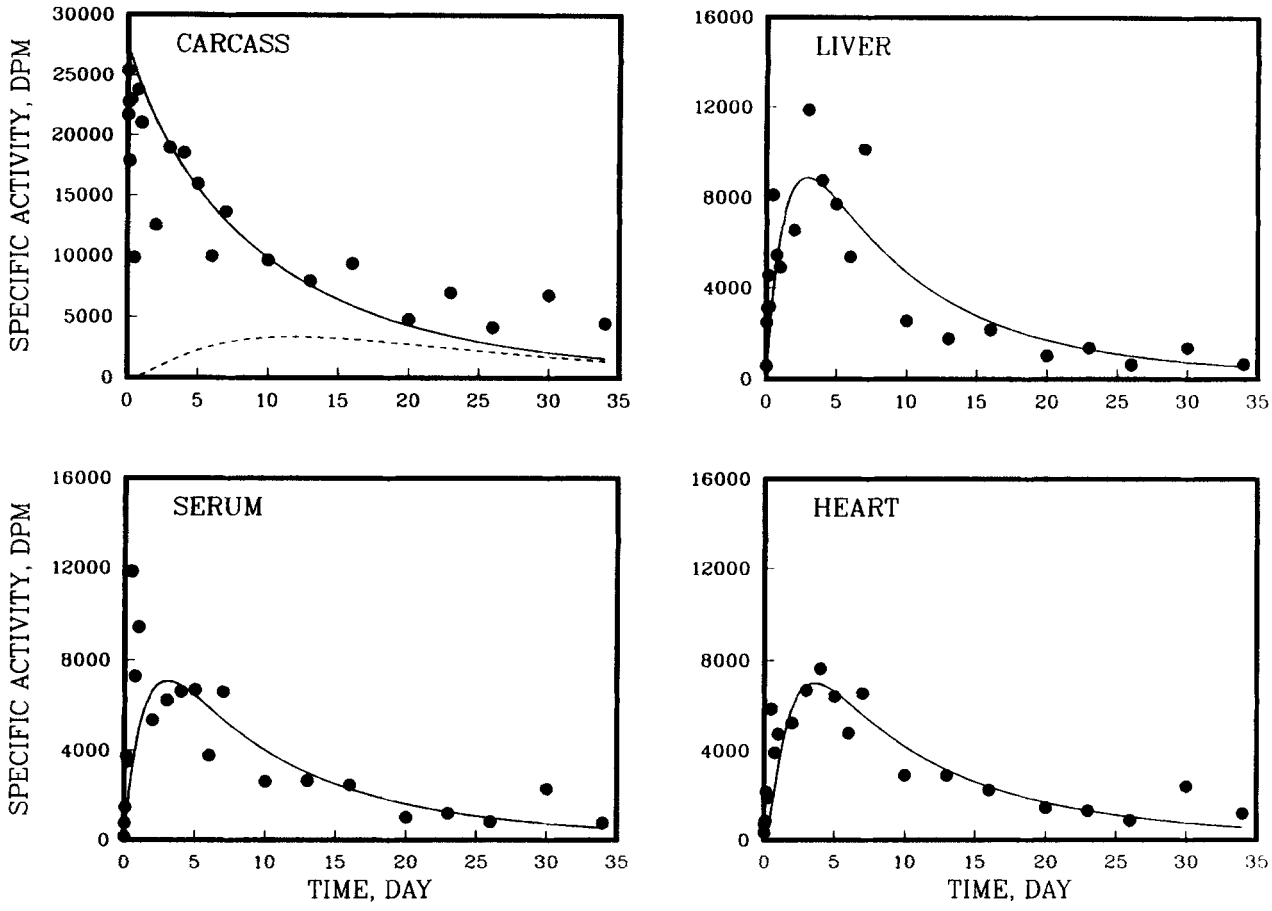

Fig. 5. Model and experimental labeled cholesterol versus time for C57BR/*cdJ* mice fed the US74 diet.

labeled cholesterol is somehow inhibited or that the serum-liver exchange flows are very rapid and the difference is a statistical result. If the former is true, then future modelling must account for the presence of the non-physiological dose in the serum compartment enroute to the liver.

The kinetic data add to the understanding of whole-body cholesterol metabolism by showing that small dietary differences can cause major changes in rates of movement of cholesterol between organs, and regulation of synthesis. Large differences found between the model-derived excretion and synthesis rates as well as the liver to serum exchange rates are associated with diet. Increased dietary saturated fatty acids and cholesterol seem to suppress the exchange of cholesterol to and from the liver, consistent with the functioning of the hepatic low-density lipoprotein receptors. Slower movement of cholesterol is associated with larger concentration in the liver and that may account for the lower rates of synthesis related to diet. The activity of the liver enzyme, hydroxylmethyl-glutarylCoA reductase has been measured for these two strains of mice fed these diets (11). For both strains an increase in the enzyme activity corresponds to an increase in the synthesis rate.

The two strains differ in serum and organ cholesterol concentrations but not in a consistent way. CBA/*J*, the resistant strain, had higher serum and carcass cholesterol, but lower liver and heart cholesterol. Liver concentration was much more responsive to diet in the CBA/*J* strain than in the C57BR/*cdJ* strain. Other diet/strain interactions were found in the serum-carcass and serum-heart rates of movement. The carcass had higher cholesterol when the US74 diet was fed to both strains compared with the MOD diet but the rate of movement was greater only in the CBA/*J* mice. The CBA/*J* mice, therefore, had a greater capacity to respond to diet. Heart cholesterol concentration was lower in CBA/*J* than C57BR/*cdJ* mice, and the CBA/*J* mice did not respond to the US74 diet by increased cholesterol movement as did the C57BR/*cdJ* mice.

In conclusion, the kinetic results are consistent with our

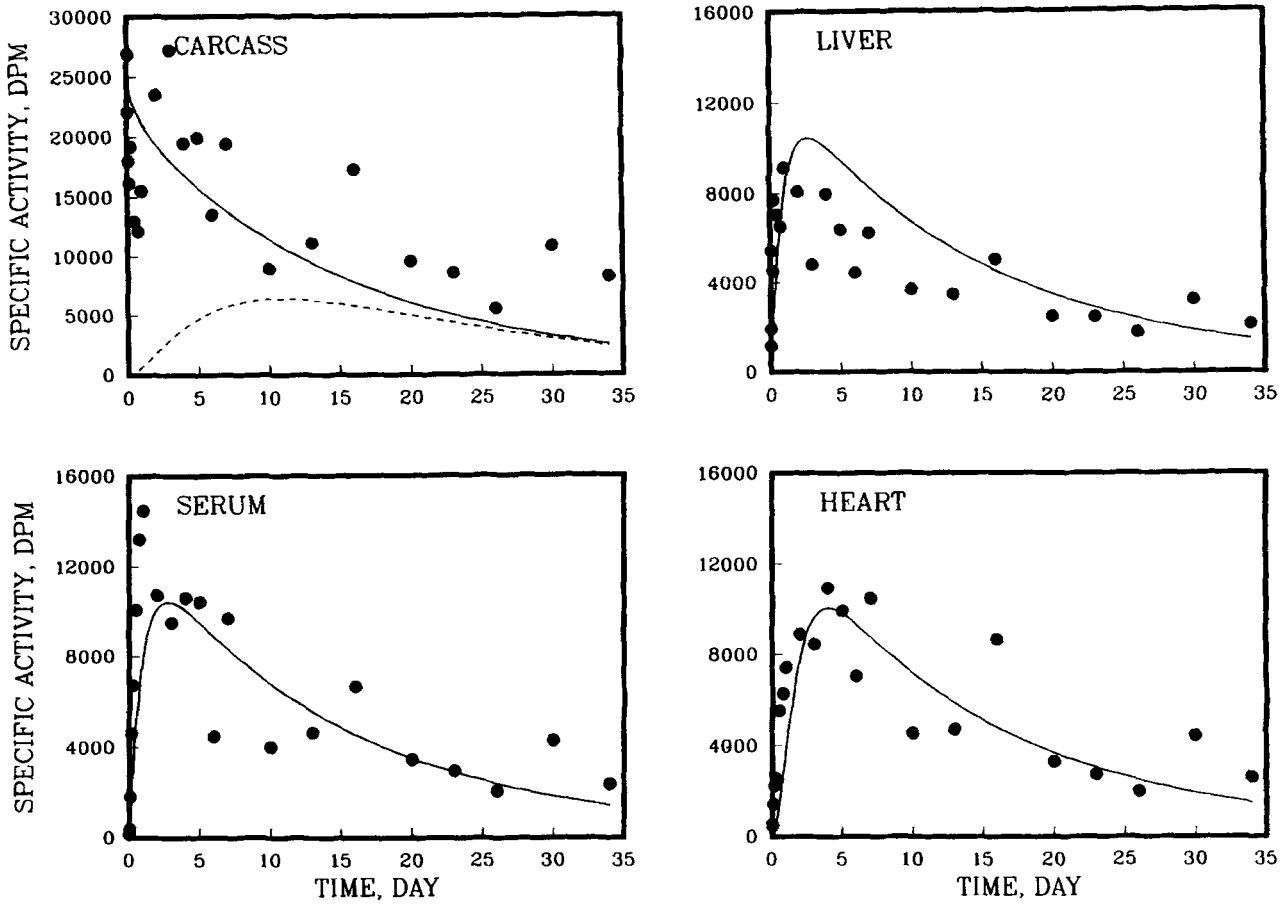

Fig. 6. Model and experimental labeled cholesterol versus time for C57BR/*cdJ* mice fed the MOD diet.

prior conclusions based upon HMGR activity and quantify the enzyme activity to show twice the dietary response in mg/h synthesis in CBA/*J* when MOD is compared with US74 diet. The excretion data derived by the kinetic analysis do not agree with previous measured sterol excretion.

Manuscript received 24 January 1992.

REFERENCES

1. Breslow, J. 1989. Genetic basis of lipoprotein disorders. *J. Clin. Invest.* **84:** 373–380.
2. Dupont, J. 1982. Cholesterol balance and whole body kinetics. *In* Cholesterol Systems in Insects and Animals. J. Dupont, editor. CRC Press, Boca Raton, FL. 117–144.
3. Clarkson, T. B., J. R. Kaplan, and M. R. Adams. 1985. The role of individual differences in lipoprotein, artery wall, gender, and behavioral responses in the development of atherosclerosis. *Ann. NY Acad. Sci.* **454:** 28–45.
4. National Cholesterol Education Program Expert Panel. 1988. Detection, evaluation, and treatment of high blood cholesterol in adults. *Arch. Intern. Med.* **148:** 36–69.
5. Paigen, B., A. Morrow, C. Brandon, D. Mitchell, and P. Holmes. 1985. Variation in susceptibility to atherosclerosis among inbred strains of mice. *Atherosclerosis.* **57:** 65–73.
6. Paigen, B., B. Y. Ishida, J. Verstuyft, R. B. Winters, and D. Albee. 1990. Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis.* **10:** 316–323.
7. Roberts, A., and J. S. Thompson. 1977. Genetic factors in the development of atheroma and on serum total cholesterol levels in inbred mice and their hybrids. *Prog. Biochem. Pharmacol.* **14:** 298–305.
8. Walker, B. L., and B. J. Mulvihill. 1984. Plasma cholesterol response to dietary saturated and hydrogenated fats in CBA and C57BR/*cdJ* mice. *Nutr. Res.* **4:** 601–610.
9. Aubert, R., D. Perdereau, M. Roubiscoul, J. Herzog, and D. Lemmonier. 1988. Genetic variations in serum lipid levels of inbred mice and response to hypercholesterolemic diet. *Lipids.* **23:** 48–54.
10. Abraham, S., and M. D. Carroll. 1979. Fats, cholesterol, and sodium intake in the diet of persons 1–74: United States. Advanced Data, Vital and Health Statistics, No. 54. U.S. Department of Health and Human Services. Washington D.C.
11. Kuan, S. I., and J. Dupont. 1989. Dietary fat and cholesterol effects on cholesterol metabolism in CBA/*J* and C57BR/*cdJ* mice. *J. Nutr.* **119:** 349–355.
12. Dietary Goals for the United States. 1977. U.S. Senate Committee on Nutrition and Human Needs. Washington D.C.

13. Berman, M., M. F. Weiss, and E. Shahn. 1962. Some formal approaches to the analysis of kinetic data in terms of linear compartmental systems. *Biophys. J.* **2:** 289–315.
14. Goldberg, I. J., S. Holleran, R. Ramakrishnan, M. Adams, R. H. Palmer, R. B. Dell, and D. J. Goodman. 1990. Lack of effect of lovastatin therapy an the parameters of whole-body cholesterol metabolism. *J. Clin. Invest.* **86:** 801–808.
15. Avigan, J., D. Steinberg, and M. Berman. 1962. Distribution of labeled cholesterol in animal tissues. *J. Lipid Res.* **3:** 216–221.
16. Folch, J., M. J. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
17. Carlson, S. E., and S. Goldfarb. 1977. A sensitive enzymatic method for determination of free and esterified tissue cholesterol. *Clin. Chim. Acta.* **79:** 575–582.
18. Hellman, L., R. S. Rosenfeld, M. C. Eidinoff, D. K. Fukushima, and T. F. Gallagher. 1955. Isotopic studies of plasma cholesterol of endogenous and exogenous origins. *J. Clin. Invest.* **34:** 48–60.
19. Nilsson, A., and D. B. Zilversmit. 1972. Fate of intravenously administered particulate and lipoprotein cholesterol in rats. *J. Lipid Res.* **13:** 32–38.
20. Box, G. E. P., and N. R. Draper. 1965. The Bayesian estimation of common parameters from several responses. *Biometrika.* **46:** 77–90.
21. Bates, D. M., and Watts, D. G. 1988. Nonlinear Regression Analysis and its Applications. Wiley-Interscience, New York. 365.