Cholesterol metabolism in groups of rhesus monkeys with high or low response of serum cholesterol to an atherogenic diet

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Abstract Cholesterol metabolism has been studied in two groups of five rhesus monkeys each that were selected from the upper and lower sextile in the distribution of serum cholesterol concentration while being fed an atherogenic diet. This diet consisted of a basal commercial monkey food supplemented with saturated fat and cholesterol. To determine differences that might be related to the difference in serum cholesterol concentration, parameters of whole body cholesterol metabolism were measured while the animals were fed the supplemented diet and again while they were fed the basal diet. Serum cholesterol and triglyceride concentrations were greater in the high-responding (HI) than in the low-responding (LO) groups during both diet periods. Percent of luminal cholesterol absorbed was also greater in the HI than in the LO group during both diet periods. The increase in fecal excretion of endogenous neutral steroids during the period when the atherogenic diet was fed was similar in the two groups; however the increase in excretion of bile acids was greater in the HI than in the LO group. The three-pool model was used for analysis of decay of serum cholesterol specific activity after a tracer pulse dose. The mean size of the total miscible body pool and of pools 1 and 3 (but not pool 2) was greater in both groups when the atherogenic diet was fed than during basal diet feeding. The distribution of the increment in total body pool between pool 1 and the peripheral pools was similar in the two groups. The distribution of the increment in cholesterol of pool 1 between serum and other tissues was also similar for the two groups. There was an indication that a constant cholesterol pool size was not attained even after feeding the atherogenic diet for 8 months. This study indicates that the difference in serum cholesterol concentration between LO and HI responders is, in part, a result of differences in rate of intestinal absorption of cholesterol.

Supplementary key words cholesterol absorption · cholesterol turnover · cholesterol excretion · bile acid excretion · compartmental analysis · cholesterol balance · three pool model

Serum cholesterol concentration varies greatly among various species of animals and among individuals of the same animal species. This variability in serum cholesterol level occurs while the animals are consuming a standardized low-fat diet or an atherogenic diet but is most obvious with the latter. Various species of primate animals are prime examples of this phenomenon. Since the mechanisms producing these differences in primate animals are also likely to operate in the human, studies in suitable primate animal models should be of value in understanding the mechanisms regulating serum cholesterol concentration in man.

This report presents the results of experiments that were conducted on two groups of rhesus monkeys selected from the upper and lower sextiles for the distribution of serum cholesterol concentration during a period when they were fed an atherogenic diet.

METHODS

Selection of animals

Thirty-seven young male rhesus monkeys weighing 5.4–6.4 kg were obtained from an animal importer who had fed the animals a diet low in fat and cholesterol for a minimum of eight weeks prior to shipment. Based on dentition (1) it was estimated that a majority of the animals were 3.5–4.5 years old, although a few may have been as young as 3 years or more than 6 years old. Throughout this study the animals were housed individually in stainless steel metabolism cages (24″ wide × 30″ deep × 36″) that were designed so liquid waste was drained away from solid waste.

After arrival in the laboratory the animals were fed a basal diet consisting of a low-fat, low-cholesterol baked primate ration (supplied by Price-Wilhoite Co., Frederick, Md.) for an additional conditioning period of 2–20 weeks. The animals were then fed an atherogenic diet in which the basal primate ration was supplemented to provide fat at 40% of calories, proteins at 15% of calories, cholesterol at 1 mg/kcal, and other ingredients as shown

TABLE 1. Composition of atherogenic diet

	Diet	
	Basal	Atherogenic
	g/kg	
Ingredients		
Basal monkey food[a]	1000	525.5
Casein[b]	0	85.0
Salt mixture (Hegsted IV)[b]	0	11.0
Vitamin mixture[c]	0	5.0
Butter (unsalted)	0	150.0
Beef tallow	0	45.0
Cholesterol (U.S.P.)[b]	0	3.5
Water	0	175.0
	mg/g	
Lipid Analysis		
Total lipid	13.	164.
Cholesterol	0.02	3.4
β-Sitosterol	0.45	0.23
	ratio	
Polyunsaturated/saturated fatty acid ratio	2.4	0.11

[a] D and G Baked Primate Ration, Price Wilhoite Co., Frederick, Md.
[b] Nutritional Biochemical Corp., Cleveland, Ohio.
[c] Teklad, Chagrin Falls, Ohio.

in **Table 1.** Lipid analysis of the basal and atherogenic diets are also shown in Table 1. One animal refused to eat the atherogenic diet and was removed from the study.

Total serum cholesterol was determined at 1, 2, 3, 4, 6, 8, 10, and 12 weeks after beginning the atherogenic diet. The mean serum cholesterol during the 12 weeks was calculated for each animal. The mean for each animal ranged from 151 to 721 mg/dl with a mean and standard deviation for the 36 animals of 431 ± 137 mg/dl. The 36 animals were ranked on this mean serum cholesterol concentration and the six animals with highest concentration (mean 619 mg/dl, range 567–721 mg/dl) and the six animals with lowest concentration (mean 199 mg/dl, range 151–230 mg/dl) were selected for use in the present study. These groups will be referred to as the high-responding group (HI) and low-responding group (LO), respectively. The 24 animals with median serum cholesterol levels were used in studies of regression of early atherosclerotic lesions (2–7).

Plan of study

The HI and LO groups were fed the atherogenic diet for a total period of 11.5 months and then were returned to the basal primate ration. Eight months after beginning the atherogenic diet each animal received 13.6 μCi of [4-^{14}C]cholesterol intravenously as a suspension in 5% glucose stabilized with 1% Tween-80.

Three 5-day samples of feces were collected at 2-week or 3-week intervals beginning 63 days after injecting the [4-^{14}C]cholesterol. Six days after the end of the last of these 5-day fecal samples the animals were returned to the basal diet. For three animals selected randomly from each group a contiguous series of fecal samples were also collected to determine excretion during the transition period from atherogenic to basal diet.

In order to obtain complete fecal collections the following procedure was used. The cages were washed just prior to the feces sampling period and were not washed again until the end of the sampling period. Three to four times each day, and just prior to feeding, any fecal matter was carefully transferred to a tared plastic container. Approximately one hour after feeding the food bin was removed and any spilled food was carefully removed from the pan. All feces were well formed, therefore there was little difficulty in separating spilled food from any feces excreted during the feeding period. At the end of the feces sampling period the animal was removed from the cage and feces (if any) that adhered to the floor bars was collected. Finally, the pan was rinsed with water to collect any residual fecal matter. With these precautions it is unlikely that more than a few percent of feces could have been lost. Although the cages used were not designed to prevent coprophagy it has been our experience that monkeys will not eat food that has dropped into the pan and become even slightly contaminated with excreta. Although it cannot be entirely ruled out, it is highly unlikely that coprophagy occurred during this study.

Five months after the animals were returned to the basal diet they were given a single meal containing known activity of [22,23-^{3}H]β-sitosterol and [4-^{14}C]cholesterol for the determination of absorption of cholesterol by the method of Borgström (8) and Quintao, Grundy, and Ahrens (9). Feces were collected in a single pool for the following 8 days.

Twelve months after the animals were returned to the basal diet a second dose of 20.2 μCi of [4-^{14}C]-cholesterol was administered intravenously to each animal. Three 5-day fecal samples were again collected at 2-week intervals beginning 63 days after the second intravenous dose of labeled cholesterol.

At various intervals throughout the study blood samples were obtained by venous puncture under sedation with phencyclidine hydrochloride following at 16-hr fast. Serum cholesterol and triglyceride concentrations and the specific activity of labeled serum cholesterol were determined at the times indicated below.

Chemical and isotopic analysis of serum and diet

Isotopic compounds used in the study were obtained from New England Nuclear Corp., Boston, Mass. Prior to use they were purified by thin-layer chromatography on silica gel G with a solvent system of 45% *n*-heptane in ethyl ether.

Total serum cholesterol concentration was determined on coded duplicated samples by the method of Abell et al. (10) and serum triglyceride concentration was determined on coded duplicate samples by the method of van Handel and Zilversmit (11). Carbon-14 activity per ml of serum was determined on an aliquot of the extract prepared for the cholesterol mass determination using liquid scintillation counting with external standardization for quench correction. Specific activity of serum cholesterol was calculated as the ratio of activity/ml to mass of cholesterol/ml. Reproducibility of serum lipid and activity determinations was determined from duplicate analyses. The coefficient of variation between replicates was ±2.3% for serum cholesterol, ±7% for serum triglyceride, and ±1.3% for serum ^{14}C activity.

Fecal samples were homogenized, and an aliquot of the homogenate was subjected to the procedure for extraction of neutral and acidic steroids of Miettinen, Ahrens, and Grundy (12) and Grundy, Ahrens, and Miettinen (13). Activity of ^{3}H and ^{14}C in the fecal steroids was determined on an aliquot of the fecal extracts. The mass of the total cholesterol and total β-sitosterol excreted in the feces was determined by gas–liquid chromatography of the trimethylsilyl ethers after thin-layer chromatographic separation of the unaltered sterol and the two bacterial conversion products (12).

Aliquots of the diet were analyzed for mass and activity of sterols using procedures analogous to those used for feces. The coefficient of variation for cholesterol or sitosterol content from batch to batch was about 10%. The coefficient of variation for the ratio of cholesterol to sitosterol was 3%.

Fasting glucose tolerance was determined during the basal diet period. Plasma glucose was determined by the method of Werner, Rey, and Wielingen (14) at 5, 15, 30, 45, and 60 min after intravenous injection of 0.5 g glucose per kg body weight. The slope of the monoexponential decay was used as the measure of glucose tolerance. Twelve serum parameters were determined (SMA 12/60 profile) seven months after beginning the atherogenic diet. These included CO_2, chloride, glucose, blood urea nitrogen (BUN), uric acid, calcium, inorganic phosphorus, total protein, albumin, total bilirubin, alkaline phosphatase, and serum glutamic oxaloacetic transaminase (SGOT). While the animals were being fed the basal diet, serum triiodothyronine (T_3) and thyroxine (T_4) concentrations were determined by commercially available methods (Tresitope for T_3, E. R. Squibb, N.Y., N.Y.; T_4 by column, Curtis Nuclear, Los Angeles, Calif.).

Analysis of data

Isotope kinetic data. Goodman and Noble (15) and Goodman, Noble, and Dell (16) have shown that in man the decay of serum cholesterol specific activity after intravenous administration of a pulse dose of labeled cholesterol can be represented as the sum of two or three exponentials; i.e.,

$$\text{Specific activity} = C_1e^{-\alpha_1 t} + C_2e^{-\alpha_2 t} + C_3e^{-\alpha_3 t},$$

where the third term may or may not be required. This expression describes the decay of activity in the central pool of a 3-pool mamillary model (16) after administration of a pulse tracer dose to the central pool. The generalized nonlinear least squares technique of Snedecor and Cochran (17) was used to determine the parameters giving the best fit of the logarithm of specific activity to the logarithm of the sum of two or three exponentials. The Fisher F statistic was used to test the decrease in the residual mean square when changing from the 2-pool model to the 3-pool model. The addition of a third term to the model caused a significant reduction ($P < 0.05$) in the residual mean square for 10 of 11 analyses on the test diet and for all 12 analyses during the basal diet period; therefore the 3-pool model was used for analysis of all data. The value, s, of the square root of the mean squared deviation of data points about the fitted line (3-pool) ranged from 1.1 to 3.5% of predicted value with a mean of 2.5%. Values of production rate, pool size, and rate constants for exchange between pools were calculated from the parameters of the three terms as described by Goodman et al. (16).

The results of the least squares analysis described above were found to be nearly identical with those obtained using the weighted least squares method of Dell et al. (18). Using the latter method it was also found that the addition of a fourth exponential (fourth pool) to the model did not result in a statistically significant reduction in the residual sum of squares for any of the 23 sets of data.

Values for the standard error of estimate of the model parameters, C_i and α_i, and for values derived from the model parameters were obtained using the Gauss multipliers (17). These values were found to be nearly identical to measures of standard error obtained on least squares analysis of 50 sets of data generated by adding, to the model given in the equation above, an error term that was drawn from a ran-

dom normal population with a mean of zero and variance equal to the residual variance of the experimental data.

Cholesterol absorption. The method for calculating the percentage of dietary cholesterol absorbed during the atherogenic diet period has been described previously (19). Briefly, the cholesterol that was ingested in those meals contributing unabsorbed dietary sterol directly to a fecal pool is determined as the product of the β-sitosterol content of that fecal pool and the ratio of cholesterol to β-sitosterol in the diet. The dietary cholesterol absorbed is determined as the difference between ingested cholesterol and unabsorbed cholesterol, where the latter is calculated as the difference between total cholesterol and endogenous cholesterol in the feces (20). As used in this paper the term "endogenous cholesterol" refers to that fraction of the fecal cholesterol measured by the isotopic balance procedure, i.e., that derived from sources in equilibrium with serum cholesterol. It was determined as the ratio of ^{14}C activity in fecal neutral sterol to the specific activity of ^{14}C in serum cholesterol 2 days prior to the midpoint of the fecal pool.

If it is true for the rhesus monkey, as it is for the rat (21), that a fraction of the cholesterol synthesized in the intestinal wall appears in the lumen before equilibrating with the serum cholesterol, then the mass of unabsorbed dietary cholesterol will be overestimated and the cholesterol absorbed will be underestimated by the method just described. Since the amount by which total fecal neutral sterol (determined by gas–liquid chromatographic analysis) exceeds the endogenous neutral sterol (determined by isotopic balance) during the cholesterol-free diet period represents at most only a few percent of unabsorbed dietary cholesterol during the atherogenic diet period, it is doubtful that direct excretion of newly synthesized cholesterol could have a significant effect on the measurements made here.

In this study no corrections were made for fecal flow rate or for potential degradation of steroid nucleus on transit through the intestines (20). The method outlined above for calculation of absorption during the atherogenic diet period is independent of such errors. Errors in endogenous excretion due to variability in fecal flow are minimized here by averaging over several 5-day collection periods. Denbesten et al. (22) have shown that degradation of sterol nucleus that occurs with liquid formula diets in some human subjects was greatly reduced by the addition of cellulose. Since the diet used here was based on natural sources high in fiber content, it is unlikely that appreciable degradation occurred.

During the basal diet period the percent of luminal cholesterol absorbed was determined as the percent decrease in the ratio of ^{14}C to ^{3}H on passage through the digestive tract after feeding the single meal labeled with [22,23-^{3}H] β-sitosterol and [4-^{14}C]cholesterol (8, 9).

Statistical analysis. Statistical significance of differences between HI and LO groups during each diet period were determined by *t* test of differences between means. Statistical significance of the effect of change in diet was determined by *t* test of the mean difference within each group, considering the two values obtained on each animal as paired observations (23).

RESULTS

Condition of animals

One animal in the LO group was found to be distinctly hyperthyroid by analysis of T_3 uptake and T_4 concentration and was removed from the study. Another animal in the HI group suffered a severe injury during the initial isotope kinetic study and, since the data could not be analyzed, this animal was also removed from the study. Thus data to be presented here will be based on five animals from each group.

All ten remaining animals appeared to be in excellent health throughout the study. There were no significant abnormalities in any of the clinical parameters measured in the SMA 12/60 profile or in the measures of glucose tolerance, nor were significant differences observed between HI and LO groups for any of these parameters. All animals maintained normal growth. The mean body weights at various times during the study are given in **Table 2.** No significant difference in body weight developed between groups at any time.

Serum lipids

The data on total serum cholesterol concentration are given in **Table 3.** After beginning the atherogenic diet, serum cholesterol increased in all animals, rapidly at first and then more slowly. The mean serum cholesterol concentration for the HI and LO groups remained essentially constant from the 12th to the 50th week of the atherogenic diet period; however, in some animals there was a trend toward a gradual increase or decrease in concentration. The mean concentration of serum cholesterol for 27 samples taken during this 38-week period was nearly three-fold greater in the HI (697 mg/dl) than in the LO group

TABLE 2. Mean and standard error of mean body weight by group

Group		Diet Period: Atherogenic 0 wk	Atherogenic 12 wk	Atherogenic 50 wk	Basal 22 wk	Basal 52 wk	Basal 78 wk
		kg				*kg*	
High-responding group (N = 5)	Mean	6.1	6.6	7.7	8.4	9.8	9.7
	SEM	± 0.5	± 0.5	± 0.5	± 0.6	± 0.7	± 0.8
Low-responding group (N = 5)	Mean	6.5	6.8	8.0	9.0	9.5	9.5
	SEM	± 0.5	± 0.5	± 0.5	± 0.7	± 0.9	± 0.8

(256 mg/dl). Variability from sample to sample during this period was also greater for animals in the HI group than in the LO group, so that the coefficient of variability was similar in the two groups (8–18% for the different animals).

On transition to the basal diet after feeding the atherogenic diet for 50 weeks, the serum cholesterol concentration dropped rapidly at first and then more slowly. Whereas animals in the LO group attained a steady serum cholesterol concentration in 2–3 weeks, animals in the HI group required 7–14 weeks to reach a steady concentration.

Serum cholesterol concentration of the HI group also exceeded that for the LO group during the basal diet period. Although there was a slight overlap in the ranges of serum cholesterol concentration for the two groups while they were fed the basal diet, the difference of 43 mg/dl between means was statistically significant by t test ($P < 0.02$).

The mean serum triglyceride concentrations were determined for the initial sample and for seven samples taken during each diet period. For 14 of the 15 determinations the HI group had higher mean serum triglyceride concentration than the LO group. In both HI and LO groups the mean serum triglyceride concentration was higher during the atherogenic diet period (51 and 44 mg/dl in HI and LO groups, respectively) than during the subsequent basal diet period (48 and 34 mg/dl in HI and LO groups, respectively). An analysis of variance was used to test the significance of these differences. Both the difference between groups and the difference between diets were statistically significant ($P < 0.01$). Although the difference between atherogenic and basal diets appeared to be less for the HI than for the LO group, the interaction term in the analysis of variance was not statistically significant.

Agarose gel electrophoresis of serum samples obtained near the end of the atherogenic diet period and again after the animals had been on the basal diet for many months indicated that there was a very large increase in β lipoproteins relative to α and pre-β lipoproteins during the period when the atherogenic diet was fed. The electrophoretic pattern of the two groups differed primarily in the magnitude of this increase in the β-migrating band, with the HI group having a much greater increase than the LO group. The large excess of cholesterol in the HI group is therefore probably carried in the β-migrating band. During atherogenic diet, animals in the LO group in

TABLE 3. Mean and standard error of mean total serum cholesterol for five animals of each group during various diet periods

		Diet Period: Atherogenic Initial	Atherogenic 0–12 wks	Atherogenic 13–50 wks	Basal 2 wks	Basal 8 wks	Basal 52–76 wks
No. Samples per Animal		1	8	27	1	1	22
			mg/dl			*mg/dl*	
High-responding group	Mean	139	619	697	294	155	137
	SEM[a]	± 11	± 27	± 36	± 33	± 19	± 12
Low-responding group	Mean	96	199	256	110	88	94
	SEM	± 5	± 14	± 32	± 6	± 6	± 6

[a] SEM is standard error of the mean of the five animal means for each period.

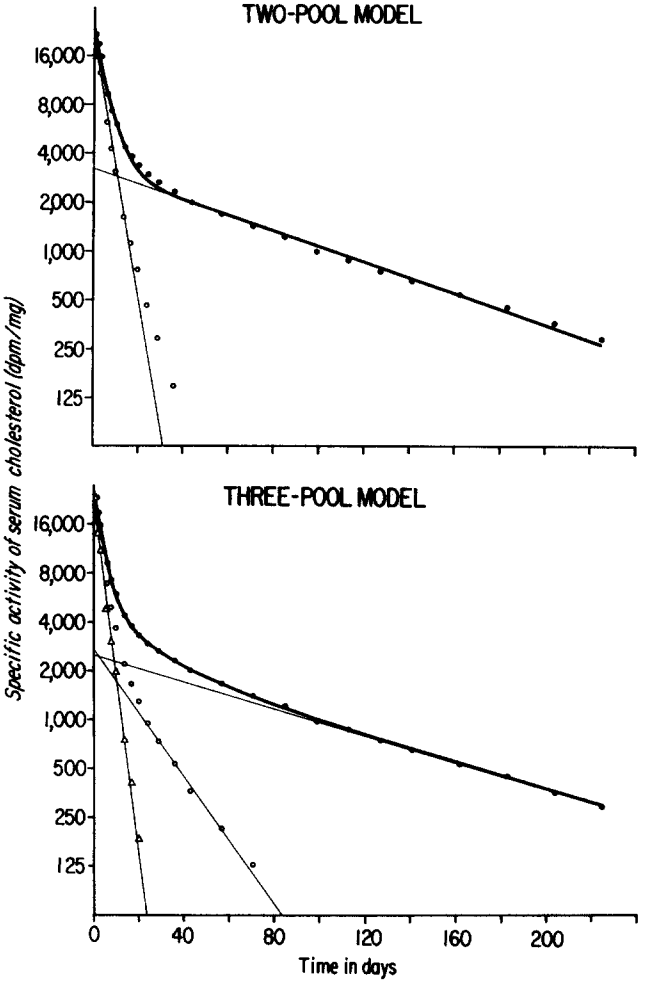

Fig. 1. Two-pool and three-pool analysis of decay of specific activity of serum cholesterol after intravenous administration of a tracer dose of [4-^{14}C]cholesterol to a rhesus monkey from the low-responding group during the basal diet period. The closed circles indicate the measured values. The open circles show the difference between measured values and extrapolation of the linear portion of the curve formed by the measured values. The triangles show the differences between the open circles and the extrapolation of the linear terminal portion of the curve formed by the open circles.

general had a slightly more intense α-migrating band than animals in the HI group.

Kinetic analysis of serum [4-^{14}C]cholesterol specific activity

Fig. 1 shows graphically the computer-derived resolution of the decay of the specific activity of [4-^{14}C]cholesterol in the serum of one animal using the two-pool and three-pool models. The improvement in fit to the data on adding the third pool to the model is readily apparent.

The mean values of the parameters of cholesterol metabolism derived from the 3-pool analysis are given in **Table 4.** The total pool size (M_{tot}) was similar in the two groups during the basal diet. The magnitude of M_{tot} was greater in both groups during atherogenic than during basal diet periods; however the difference was considerably greater for the HI than for the LO group. The fraction of the increase in M_{tot} that appeared in the rapidly exchanging pool (M_1) was approximately 50% for both groups. Using a serum volume of 45 ml/kg body weight and the mean value of serum cholesterol concentration (Table 3), it can be shown that the fraction of the absolute increase in M_1 that appeared in the serum was similar for the HI and LO groups (60 and 65%, respectively).

The mean size of pool 3 was also greater in both groups during atherogenic than during basal diet but this difference was statistically significant only for the HI group. For both groups the mean size of pool 2 was lower during atherogenic than during basal diet, however these differences were not statistically significant. That the size of pool 2 is apparently independent of serum cholesterol concentration is consistent with the observations of Smith et al. (24) in the human.

During the basal diet period the total rate of transfer of cholesterol out of pool 1, 2, or 3 (Q_{ii}) was similar for the two groups. In both groups the rates were greater during atherogenic than during basal diet. The total flux of cholesterol out of pool 1 during atherogenic diet was greater in HI than in LO groups ($P < 0.05$).

Production rate for pool 1 (PR_1) decreased on transition to basal diet. This decrease was greater for the HI (29–10 mg/day per kg) than for the LO (23–11 mg/day per kg) group, and this difference between groups in change of PR_1 was statistically significant ($P < 0.01$).

Isotopic and chemical analyses of feces

Transition to basal diet. **Fig. 2** shows the mean fecal excretion of endogenous neutral steroid and bile acid in those three animals of each group from whom fecal samples were collected during the transition period following return to basal diet. In both groups the change to basal diet resulted in a gradual decrease in rate of excretion of both neutral and acidic steroid fractions. Although a new steady state for fecal excretion had not been reached in the 9 weeks during which continuous fecal samples were obtained, there is an indication that the decline to a new constant rate of excretion was more rapid for the LO than for the HI group.

Endogenous excretion. The mean data obtained from the fecal analyses for the five animals in each group during the period of steady state for fecal excretion

TABLE 4. Mean and standard error of differences (SE_{diff}) for parameters of 3-pool model for cholesterol metabolism determined by analysis of decay of specific activity of [4-^{14}C]cholesterol in serum after intravenous administration of a pulse tracer dose to groups of five high-responding (HI) and five low-responding (LO) rhesus monkeys while they were fed atherogenic diet and while they were fed basal diet

		Diet						Standard Error and Statistical Significance of Mean Difference between Diets[b]	
		Atherogenic			Basal				
Parameter[a]	Units	HI Group	SE_{diff}[c]	LO Group	HI Group	SE_{diff}[c]	LO Group	HI Group	LO Group
M_1	mg/kg	618	44†	288	202	13	176	36†	27*
M_2 (min)	mg/kg	237	39	152	254	41	218	53	49
M_3 (min)	mg/kg	794	111*	512	312	76	336	124*	70
M_{tot} (min)	mg/kg	1649	111†	952	769	68	730	105†	68*
Q_{11}	mg/day/kg	91.7	5.8*	75.3	34.1	1.6	33.4	7.5†	3.1†
Q_{22} (min)	mg/day/kg	35.6	4.8	32.2	16.9	2.0	14.9	4.6†	3.9†
Q_{33} (min)	mg/day/kg	26.9	4.0	19.9	7.0	2.1	7.4	4.6†	3.1*
PR_1	mg/day/kg	29.4	1.3†	23.2	10.2	0.9	11.2	1.1†	1.1†

[a] Definition of symbols: M_i, mass of cholesterol in pool i; M_{tot}, sum of the three M_i; PR_1, production rate for the rapidly miscible pool (pool 1); Q_{i1}, total mass of cholesterol transferred out of pool i per day. Only minimum values for mass of pools 2 and 3 are given and are based on the assumption that cholesterol synthesis in pools 2 and 3 represent only a negligible fraction of total body synthesis of cholesterol.

[b] Statistical significance determined by t test of mean of differences between diets for each animal (*, $t > 2.77$ or $P < 0.05$; †, $t > 4.60$ or $P < 0.01$).

[c] Statistical significance determined by t test of difference between means (*, $t > 2.31$ or $P < 0.05$; †, $t > 3.36$ or $P < 0.01$).

on each diet are given in **Table 5.** In both groups the rate of fecal excretion of endogenous neutral steroids was greater by a factor of about three on atherogenic diet than on basal diet. On either diet endogenous neutral steroid excretion was similar for the two groups. During the atherogenic diet period, the fecal excretion of bile acids was greater for the HI than for the LO group; however during the basal diet period, bile acid excretion was similar in the two groups. Thus the decrease in bile acid excretion on transition to basal diet was greater for the HI than for the LO group.

In both HI and LO groups the mean total endogenous steroid excretion was greater during the atherogenic than during the basal diet period. The mean value for total fecal excretion was greater in the HI than in the LO group during atherogenic diet ($P < 0.05$). The difference between groups in total endogenous excretion during basal diet was not statistically significant. The total endogenous excretion determined by isotope balance is 20–25% lower than the production rate (Table 4). This difference may reflect losses from the body pool via pathways other than fecal excretion. In the human appreciable losses have been shown to occur through the skin (25).

Absorption of cholesterol. The rate of cholesterol ingestion (Table 5) was similar in the two groups. The rate of absorption of dietary cholesterol was greater in the HI than in the LO group. The percentage of luminal cholesterol absorbed in the HI group exceeded that in the LO group by 7 and 14% on atherogenic and basal diets, respectively. These differences between groups were statistically significant ($P < 0.01$). The increase in percent of luminal cholesterol absorbed with transition to basal diet was statistically significant for the HI ($P < 0.05$) but not for the LO group. The HI group absorbed an average of 6.1 mg/day per kg more dietary cholesterol than the LO group. This difference represents about 25%

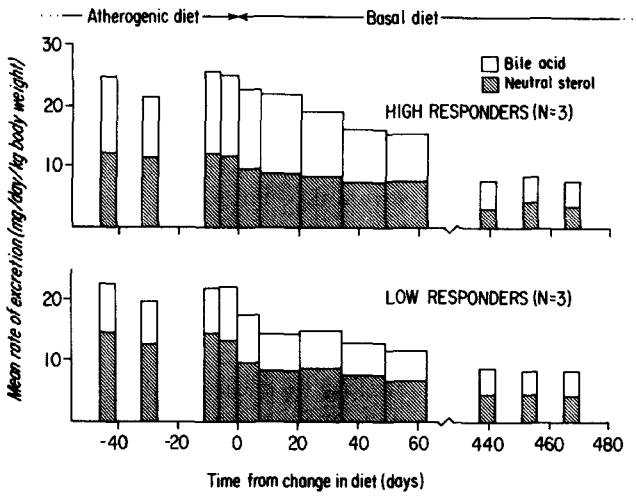

Fig. 2. Mean rate of fecal excretion of endogenous neutral steroid and bile acid in 3 high-responding and 3 low-responding rhesus monkeys while they were fed atherogenic and basal diets, and change in rate of excretion during 9 weeks following change from atherogenic to basal diet. Here, "endogenous" refers to that fraction of fecal steroid derived from sources in isotopic equilibrium with serum.

TABLE 5. Mean data derived from isotopic balance determinations in five high-responding (HI) and five low-responding (LO) rhesus monkeys during atherogenic and basal diet periods

Parameter	Units	Diet: Atherogenic HI Group	Atherogenic SE diff[b]	Atherogenic LO Group	Diet: Basal HI Group	Basal SE diff[b]	Basal LO Group	Standard Error and Statistical Significance of Mean Difference between Diets[a]: HI Group	LO Group
Endogenous neutral steroid in feces	mg/day/kg	12.2	1.2	13.1	3.8	0.6	4.6	0.88†	0.85†
Acidic steroid in feces	mg/day/kg	11.3	0.9†	7.0	3.8	0.4	3.8	0.85†	0.54†
Total endogenous fecal steroid excretion	mg/day/kg	23.5	1.4*	20.1	7.6	0.8	8.4	1.02†	1.26†
Cholesterol ingested	mg/day/kg	66.3	6.7	63.3	0		0		
Dietary cholesterol absorbed	mg/day/kg	35.0	2.6*	28.9	0		0		
Percent cholesterol absorbed	percent	52.9	1.8†	45.4	59.7	2.7†	46.2	1.2†	1.2
Total cholesterol absorbed	mg/day/kg	49.3	4.0*	40.1	7.3	0.7†	4.7	3.5†	2.8†
Total luminal cholesterol flux	mg/day/kg	92.7	7.3	87.6	12.1	1.4	10.3	6.1†	4.3†

[a] Values indicate standard error of mean difference. Statistical significance of mean difference between diets determined by t test of mean differences. (*, $t > 2.77$, $P < 0.05$; †, $t > 4.60$, $P < 0.01$).

[b] Values indicate standard error of difference between means. Statistical significance of the difference between means for groups determined by t test. (*, $t > 2.31$, $P < 0.05$; †, $t > 3.36$, $P < 0.01$).

of the mean production rate for the rapidly miscible pool (Table 4).

The value of total luminal flux of cholesterol (Table 5) was calculated as the ratio of total fecal neutral sterol to the fraction of luminal cholesterol not absorbed. The total rate of absorption of cholesterol was then obtained as the product of total luminal flux and the fraction of luminal cholesterol absorbed. The mean total luminal cholesterol absorbed in the HI group exceeded that in the LO group by 9.2 mg/day per kg during atherogenic diet and by 2.6 mg/day per kg during basal diet.

The data on cholesterol balance indicate that a steady state for body pool size may not have been attained even after the animals had eaten the atherogenic diet for 8 months. Thus, the calculated rate of absorption of dietary cholesterol exceeds endogenous excretion by 12 and 18 mg/day per kg. Since it is known (26) that even when very high cholesterol diets are fed there is an appreciable residual synthesis of cholesterol, the discrepancy between total input and loss of cholesterol is likely to be even larger than these differences indicate. A fraction of this difference between total cholesterol input and losses in feces can be accounted for by losses of cholesterol through the skin (25) and by increase in total body cholesterol due to the mean growth rate of approximately 4 g/day. It is likely however that an appreciable positive balance remains and that cholesterol may have continued to accumulate in peripheral tissues.

The effects of such deviation from the assumed condition of steady state on the parameters derived from the isotope kinetic analysis have not been determined. The small values for the mean deviation about the fitted curve that we observed (1–3%) and the fact that these deviations appeared to be uniformly and haphazardly distributed throughout the duration of the study would indicate that the effect of this failure to attain true steady state was not large. Since any resulting bias is likely to have affected both groups, it is unlikely that it would have appreciably affected the conclusion that increased body cholesterol was distributed similarly in HI and LO groups.

DISCUSSION

In this study we have examined whole-body cholesterol metabolism in groups of rhesus monkeys differing greatly in response of serum cholesterol concentration to an atherogenic diet. Our objective was to determine how the cholesterol metabolism of these groups may differ so as to cause the large difference in serum cholesterol concentration. We studied cholesterol metabolism while the animals were fed the atherogenic diet and again while they were fed a commercial monkey food. In addition to serum concentration we determined (*1*) the rate of fecal excretion of cholesterol and bile acids, (*2*) the fraction of luminal cholesterol that was absorbed, (*3*) the

rate of cholesterol absorption, (4) the total turnover rate of cholesterol, (5) the size of the total body pool of cholesterol, (6) the distribution of cholesterol between serum, other components of the central pool, and peripheral pools, and (7) the fluxes of cholesterol between the pools.

After return to the basal diet, serum cholesterol concentration remained greater in the HI than in the LO group. Among those aspects of cholesterol metabolism examined, the only difference between HI and LO groups that might explain some of the difference in concentration of serum cholesterol was in the absorption of luminal cholesterol.

During the atherogenic diet period the HI group absorbed an average of 9 mg/day per kg more of luminal cholesterol than the LO group. Approximately 66% of this difference was contributed by dietary cholesterol. During both diets the rate of reabsorption of cholesterol excreted in the bile was greater in the HI than in the LO group by about 3 mg/day per kg. The difference in dietary cholesterol absorbed represents approximately 25% of the turnover rate PR_1.

The fraction of the 3-fold difference in serum cholesterol concentration that can be explained by a 20–25% difference in absorption has not been determined. If it is assumed that a linear relationship exists between the mass of cholesterol in the central (rapidly miscible) pool and the production rate or turnover of this pool, then it is clear that only a small fraction of the difference in serum concentration or pool size can be explained by difference in absorption. The functional relationship between central pool size and rate of turnover or excretion is not known; however, there is no reason to assume that it is linear throughout this range nor that it is the same for all animals. Thus to increase excretion enough to balance a small increase in cholesterol input might require a proportionally much larger increase in serum concentration or pool size and the increase required might be larger for one animal than for another.

We have found that the increase in body pool of cholesterol that occurred on feeding an atherogenic diet was distributed similarly in animals of the HI and LO groups. Thus it is apparently the magnitude of the total increase in body pool of cholesterol that determines the extent of increase of serum cholesterol concentration and not a difference in the way this added cholesterol is distributed among various compartments.

Lofland et al. (27) reported that for the squirrel monkey a difference in ability to alter the rate of catabolism of cholesterol to bile acid may be one reason for the lower serum cholesterol concentration in the low-responder. This differs from the results in the present study in rhesus, where we found that the amount by which bile acid excretion during atherogenic diet exceeded that during basal diet was greater for the HI than for the LO group.

In their initial study Lofland et al. (27) did not show significant differences in absorption of cholesterol between high- and low-responding squirrel monkeys. In a later study, however, Jones et al. (28) concluded that cholesterol absorption was significantly greater in high- than in low-responding squirrel monkeys. The differences between the findings in the present study and those obtained in squirrel monkeys may indicate basic differences in regulation of serum cholesterol concentration in the two species. They may, however, only reflect differences in experimental conditions used in the two studies.

It is likely that factors not examined here also play a role in producing the difference in serum cholesterol concentration between HI and LO groups. Differences in rate of catabolism or production of low density lipoproteins or differences in the rate of cholesterol synthesis or in the regulation of cholesterol synthesis may be involved. It is clear that a complete understanding of the reasons for the large differences in response of serum cholesterol to variations in dietary cholesterol will not be possible until all aspects of the mechanism for homeostasis of serum cholesterol or lipoprotein concentrations are known. Nevertheless the results obtained here do indicate that variations in rate of absorption of cholesterol in the intestines is one of the factors involved in determining serum cholesterol concentration. We do not yet know at what stage in the process of intestinal absorption of cholesterol these differences among animals occur. They may reflect differences in rates of one or more of the biochemical reactions involved in the absorption process, differences in surface area of the intestinal lumen, or differences in motility of the intestine.

Studies by Clarkson et al. (29) in the squirrel monkey and by others in a variety of species (30–34) have indicated that there is an appreciable heritable component to the variation in serum cholesterol concentration among individual animals. It is likely that an appreciable fraction of the apparently normally distributed variability in response of rhesus monkeys (35) is genetically determined and it is probable that the mode of inheritance is polygenic.

Goldstein et al. (36, 37) have shown that the lipid abnormality in a large fraction (30%) of the hypercholesteremic survivors of myocardial infarction can best be interpreted as having a polygenic mode of inheritance. The remainder are apparently hyper-

cholesteremic because of monogenically inherited defects. It may be that in man, as in the rhesus monkey, an appreciable fraction of the variability in serum cholesterol is a result of variations in intestinal absorption of cholesterol. If so, a better understanding of the source of the biochemical or physical locus involved in producing the variations in absorption in the rhesus might provide a basis for treatment of those hypercholesteremic patients whose elevated serum cholesterol concentration is not explainable by the monogenically inherited abnormalities (37).

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