Cholesterol metabolism in rhesus monkey, squirrel monkey, and baboon

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Abstract The metabolism of cholesterol was studied in baboons, rhesus monkeys, and squirrel monkeys while they were being fed either a low fat, low cholesterol (basal) diet or the basal diet supplemented with saturated fat and cholesterol (atherogenic diet). When the diet was changed from basal to atherogenic, the mean total serum cholesterol concentration increased from 70 to 180 mg/dl in the baboon, from 168 to 283 mg/dl in the squirrel monkey, and from 144 to 608 mg/dl in the rhesus monkey. In animals fed the atherogenic diet, the percentage of dietary cholesterol absorbed was greatest in the rhesus monkey and least in the baboon. The fraction of the total body pool of cholesterol that was derived from the diet was greatest in the squirrel monkey and least in the baboon. The turnover of the body pool of cholesterol was several times faster in the squirrel monkey than in the baboon or the rhesus monkey when either dict was fed. The mean total fecal excretion of endogenous cholesterol and bile acid increased in all species on transition to the atherogenic diet; however, the relative contributions of the neutral and acidic fractions to the increase in total excretion differed among species. The difference in percentage of dietary cholesterol absorbed may, in part, account for the large differences in serum cholesterol during the atherogenic diet period. Comparison with other published results indicates that of these species cholesterol metabolism in the baboon is most like that in the human.

Supplementary key words cholesterol absorption · cholesterol excretion · cholesterol turnover · bile acid excretion

Investigators have used a number of different species of subhuman primates for experimental studies of atherosclerosis. The usual practice has been to feed an atherogenic diet until hyperlipidemia and lipid-containing arterial lesions develop. In these studies the extent of hypercholesteremia produced varied widely among species; however, the differences in metabolism of cholesterol responsible for this variability have not been determined.

We have used the combined methods of isotopic balance and fecal sterol analysis described by Grundy and Ahrens (1) to determine some parameters of cholesterol metabolism in rhesus monkeys, squirrel monkeys, and baboons while they were fed basal chow and again while they were fed an atherogenic diet. This paper presents the results of this study.

METHODS

Animals

Eight baboons, eight rhesus monkeys, and five squirrel monkeys were obtained through animal importers[1] who had fed the animals a low fat diet for at least 8 wk prior to shipment. To minimize effects of variability in age and sex, we specified that the animals be young adult males, i.e., just postpubertal. The selection of animals by the importers was based on general appearance, secondary sexual characteristics, and body weight. The animals were in good health on arrival and remained in good health throughout the study. All animals were caged individually, the baboons in cages 30 × 30 × 44 inches, the rhesus monkeys in cages 30 × 30 × 32 inches, and the squirrel monkeys in cages 24 × 24 × 27 inches.

The initial mean weights of the animals were 20.8 kg for baboons, 4.1 kg for rhesus, and 0.64 kg for squirrel monkeys. Based on published data for average adult body weight of these species, it is probable that the rhesus monkeys were younger than the baboons and squirrel monkeys. During the measurement of cholesterol metabolism in animals fed the basal diet, the mean rates of increase in body weight were 5 g/day, 4 g/day, and 0.3 g/day for baboon, rhesus monkey, and squirrel monkey, respectively. During the cholesterol metabolism studies when the animals were fed the atherogenic diet, the corresponding rates of growth were 0.0, 3.0, and 0.1 g/day, respectively.

Diets

Upon receipt, the animals were fed ab lib. the basal diet, a commercial primate ration (D & G baked primate

[1] Baboons (*Papio cynocephalus*) were obtained from the Southwest Foundation for Research and Education, San Antonio, Texas. Rhesus monkeys (*Macaca mulatta*) were obtained from Shamrock Farms, Middletown, N. Y. Squirrel monkeys (*Saimiri sciureus*) were obtained from the Tarpon Springs Zoo, Tarpon Springs, Fla.

TABLE 1. Composition of atherogenic diet

Ingredient	g/kg of diet
Monkey chow[a]	525
Casein[b]	85
Salt mixture (Hegsted IV)	11
Vitamin mixture[c]	5
Butter (unsalted)	150
Beef tallow	45
Cholesterol (U.S.P.)[b]	3.5
Water	175

[a] D & G baked primate ration, Price-Wilhoite Co., Frederick, Md.
[b] Nutritional Biochemicals Corp., Cleveland, Ohio.
[c] General Biochemicals, Chagrin Falls, Ohio.

ration supplied by Price-Wilhoite Co., Frederick, Md.) that, according to data from the manufacturer, contained 2% fat and 18% protein. After 7–8 months the animals were fed an atherogenic (test) diet consisting of the primate ration mixed with fat, casein, cholesterol, vitamins, salts, and water in the proportions shown in Table 1. This diet was formulated to provide 40% of calories as fat and to maintain 18% of calories as protein while providing cholesterol at a level of 1 mg/kcal. Lipid analyses of basal and supplemented diets are given in Table 2. During the atherogenic diet period, a weighed amount of food was offered to each animal once a day. Although most animals consumed the full ration quickly, no attempt was made to quantitate accurately the amount of food ingested.

Experimental procedure

After the animals had been maintained on the basal diet for a minimum of 2 months, a tracer dose of [4-^{14}C]cholesterol stabilized in 5% dextrose with 1% Tween-80 was injected intravenously into each of the animals while they were under sedation with phencyclidine hydrochloride (Sernylan; Parke, Davis & Co.). 5 months later the animals were placed on the atherogenic diet. 1 month after beginning the atherogenic diet, tracer amounts of [1,2-^{3}H]-cholesterol were added to the diet to attain a specific activity of 2080 dpm/mg. This labeling was continued for 4.5 months. 3 months after beginning the test diet, a second injection of [4-^{14}C]cholesterol was administered.

Serum samples were taken at 2–3-wk intervals throughout the study. The animals were fasted for 16 hr and blood was obtained while the animals were under sedation with phencyclidine hydrochloride. The sera were frozen and stored at −10°C for subsequent lipid or radioisotope analyses.

2 months after each injection (i.e., during the period of log-linear decay of serum [4-^{14}C]cholesterol specific activity) a 5- or 7-day fecal sample was collected. The feces were homogenized and an aliquot was frozen and stored for subsequent analysis.

TABLE 2. Lipid analysis of basal and atherogenic (test) diets

Component	Diet: Basal	Diet: Test
Total lipid (mg/g)	13	164
Cholesterol (mg/g)	0.017	3.4
β-Sitosterol (mg/g)	0.45	0.23
Fatty acids (% of total)		
12:0[a]	tr[b]	2.23
14:0	tr	9.8
16:0	21.8	31.8
16:1	tr	1.4
18:0	1.3	17.9
18:1	18.8	30.4
18:2	54.2	6.5
18:3	3.9	0.4

[a] Number of carbon atoms:number of double bonds.
[b] tr, trace (<0.5%).

Chemical and isotopic analyses

Total serum cholesterol concentration was determined on coded duplicate aliquots by the method of Abell et al. (2). Day-to-day variability was monitored by inclusion of duplicate aliquots of a single serum pool in each day's analyses. ^{3}H and ^{14}C activities of serum cholesterol were determined on an aliquot of the extract prepared for the cholesterol mass determination. Radioactivity was determined by liquid scintillation counting using a Packard Tri-Carb model 3314 liquid scintillation counter with external standardization for quench correction. Reproducibility of the serum cholesterol concentration and specific activity determinations was calculated from the duplicate analyses. Relative error of replication was ±2% for serum cholesterol concentration and ±1.5% for serum cholesterol ^{14}C and ^{3}H activity.

Neutral and acidic steroids were extracted from an aliquot of the fecal homogenate by the procedures of Grundy, Ahrens, and Miettinen (3) and Miettinen, Ahrens, and Grundy (4). ^{14}C in the neutral and acidic steroids was determined by counting an aliquot of the extracts. Mass of total fecal sterols was determined by gas–liquid chromatography after thin-layer chromatographic separation of the unaltered sterol and the two major bacterial conversion products (4). In the following, the term neutral sterols will be used to refer to the sum of cholesterol and its bacterial conversion products, coprostanol and coprostanone, and will exclude all plant sterols.

Analysis of data

Serum cholesterol specific activity was calculated as the ratio of activity/milliliter to mass/milliliter. The specific activity of ^{14}C in serum cholesterol was plotted against time on semilog paper, and the period of monoexponential decay was determined. The slope of decay during this exponential phase was determined by least squares fit (5), and the half-time of decay was calculated from this slope.

TABLE 3. Mean total serum cholesterol concentrations for baboons and rhesus and squirrel monkeys fed basal and atherogenic (test) diets

	Baboon (n = 8)		Rhesus Monkey (n = 8)		Squirrel Monkey (n = 5)	
	Basal Diet	Test Diet	Basal Diet	Test Diet	Basal Diet	Test Diet
Serum samples per animal	7	16	7	17	7	17
Mean[a] (mg/dl)	70	180	144	608	168	283
SEM	3	9	8	41	4	17
Range	60–83	155–214	109–179	491–702	145–168	253–350

[a] Mean of all determinations for each animal and for all animals of each species.

The specific activity of ^{3}H in serum cholesterol was plotted against time, and the final level was determined. In order to allow for the failure of the ^{3}H specific activity to attain the asymptotic level within the 140 days during which dietary cholesterol was labeled, we applied a correction factor derived by the method of Zilversmit and Wentworth (6). Parameters used in calculating this correction factor were determined in an unpublished pilot study in which the data were analyzed by the two-pool method (7). These correction factors were +7%, +10%, and +1% for baboon and rhesus and squirrel monkeys, respectively. The ratio of this corrected asymptotic specific activity to the specific activity of the dietary cholesterol was taken to be the fraction of the serum cholesterol pool that was derived from the diet.

The contribution to the total fecal neutral sterols that was derived from cholesterol excreted in the bile, which will be referred to here as fecal endogenous neutral sterol, was determined from the ratio of fecal neutral sterol ^{14}C activity to the specific activity of ^{14}C in serum cholesterol 2 days prior to the midpoint of the fecal collection (1). The mass of fecal bile acid was determined as the ratio of activity of ^{14}C in fecal bile acids to the specific activity of ^{14}C in serum cholesterol 4 days prior to the midpoint of the fecal collection (1). The sum of fecal bile acids and fecal endogenous neutral sterol will be referred to here as total fecal excretion of endogenous cholesterol and its catabolites.

Absorption of dietary cholesterol during the test diet period was calculated by "method 1" of Grundy and Ahrens (1), in which unabsorbed cholesterol is determined by the difference between total fecal neutral sterols determined by gas–liquid chromatographic analysis and the endogenous contribution to the total fecal neutral sterols determined from activity of ^{14}C in the fecal neutral sterols. In this study the ingested cholesterol that contributed to the fecal pool was determined as the product of β-sitosterol in the fecal pool and the ratio of cholesterol to β-sitosterol in the diet (determined by gas–liquid chromatography). This method corrects for variations in fecal flow and for any degradation of neutral sterol (8) that might occur. This method does, however, assume that the excretion of β-sitosterol equals ingestion of β-sitosterol, i.e., that the difference between absorption of β-sitosterol and endogenous excretion of β-sitosterol is negligible. It has been shown that, in man, absorption of β-sitosterol does not generally exceed 5% and that the turnover of β-sitosterol is very rapid (9), so that an equilibrium between absorption and excretion should be established rapidly. Therefore, it is unlikely that appreciable error in determining ingested cholesterol is introduced as a result of absorption of β-sitosterol.

Differences in means among species were tested by analysis of variance and by the method of least significant difference (5). Differences between means on the two diets for each species were tested by t test of paired observations (5).

RESULTS

The data on total serum cholesterol are given in Table 3. The serum cholesterol of all animals was essentially constant during the basal diet period. When the basal diet was fed, the serum cholesterol was significantly lower in the baboon than in the rhesus or squirrel monkey ($P < 0.05$). Serum cholesterol increased in all animals after they were fed the test diet. The mean serum cholesterol was significantly greater during the test diet period than during the basal diet period for all species ($P < 0.01$).

Serum cholesterol concentration during the test diet period was lowest in baboons, intermediate in squirrel monkeys, and highest in rhesus monkeys. All differences in mean serum cholesterol concentration among species were statistically significant ($P < 0.01$). While measuring cholesterol metabolism during the test diet period, the total serum cholesterol in baboons and squirrel monkeys was essentially constant, with mean levels of 180 and 280 mg/dl, respectively. During the test diet period, the mean level of serum cholesterol concentration for the rhesus monkey was 608 mg/dl; however, the changes of the serum cholesterol with time were more variable in this species. On the average, serum cholesterol concentration was declining at a rate of about 1 mg/dl/day during the period when cholesterol metabolism measurements were made.

The means, standard errors, and ranges of values derived from analysis of fecal neutral sterols during the ath-

TABLE 4. Absorption of dietary cholesterol in baboons and in rhesus and squirrel monkeys fed atherogenic (test) diet

Line	Parameter	Measure	Baboon (n = 8)	Rhesus Monkey (n = 8)	Squirrel Monkey (n = 5)
1	Ingested cholesterol (mg/day)	Mean	782	323	68
		SEM	45	17	4
		Range	550–920	255–395	58–82
2	Cholesterol absorbed from diet = ingested cholesterol (line 1) minus unabsorbed cholesterol (line 4 − line 5) (mg/day)	Mean	296	157	28
		SEM	34	12	3
		Range	122–417	120–200	18–34
3	Dietary cholesterol absorbed (%)	Mean	37	48	42
		SEM	2.8	1.8	3.6
		Range	22–45	44–54	31–52
4	Total fecal neutral sterol (mg/day)	Mean	713	243	60
		SEM	20	11	5
		Range	610–770	200–300	46–76
5	Endogenous fecal neutral sterol = activity in fecal neutral sterol/serum specific activity	Mean	227	76	20
		SEM	8.0	4.6	1.8
		Range	198–275	58–98	16–24
6	Contribution of dietary cholesterol to serum cholesterol pool (%)	Mean	60	69	73
		SEM	0.8	1.4	1.3
		Range	57–63	62–77	70–78

erogenic diet period are given in Table 4. The mean percentage of ingested cholesterol that was absorbed (line 3) was greatest for the rhesus monkey and least for the baboon. Analysis of variance indicates that the difference between baboon and rhesus monkey is significant ($P < 0.02$). The difference between squirrel monkey and rhesus monkey is of borderline significance ($P < 0.10$), and the difference between baboon and squirrel monkey is not statistically significant.

The data on percentage contribution of dietary cholesterol to the serum cholesterol pool are given in Table 4 (line 6). The percentage from diet was greatest in the squirrel monkey and least in the baboon. The differences between baboon and rhesus or squirrel monkey were statistically significant ($P < 0.05$). The difference between squirrel and rhesus monkeys was not statistically significant.

A comparison of mean rates of fecal excretion of endogenous neutral sterols and bile acid in animals fed the two diets is given for each species in Table 5. When animals were changed from the basal to the test diet, endogenous neutral sterol excretion increased in all three species; however, the increase was statistically significant only in baboon and rhesus monkey. The mean rate of bile acid excretion also increased in all three species, but the increase was statistically significant only for the rhesus monkey. The increase in total endogenous excretion was statistically significant for baboon and rhesus monkey but not for the squirrel monkey. On both diets the fraction of the total endogenous excretion occurring as bile acids was less

TABLE 5. Mean rates of fecal excretion of endogenous cholesterol and bile acid and half-times of decay of serum cholesterol specific activity in baboons and in rhesus and squirrel monkeys fed basal and atherogenic (test) diets

Parameter	Measure	Baboon (n = 8)		Rhesus Monkey (n = 8)		Squirrel Monkey (n = 5)	
		Basal Diet	Test Diet	Basal Diet	Test Diet	Basal Diet	Test Diet
Endogenous fecal neutral sterol[a] (mg/day)	Mean	128	227[b]	21	76[b]	17	20
	SEM	4	8	1	5	2	2
	Range	114–140	198–275	16–30	58–96	11–20	16–24
Fecal bile acid (mg/day)	Mean	68	74	21	37[b]	16	17
	SEM	4	8	1	3	2	2
	Range	55–89	54–103	14–26	22–49	10–22	11–22
Total endogenous excretion (mg/day)	Mean	196	300[b]	42	113[b]	33	37
	SEM	7	5	2	5	2	3
	Range	175–225	288–322	30–49	90–134	28–42	27–45
Half-time for decay of serum specific activity (days)	Mean	50.2	47.0[c]	38.4	42.2	19.0	15.6[b]
	SEM	1.4	0.9	2.4	2.2	0.6	0.2
	Range	47–56	45–53	31–49	34–51	17–20	15–16

[a] Endogenous fecal sterol refers to that fraction of fecal sterol that is derived from cholesterol excreted in the bile or sloughed off from intestinal mucosa.

[b,c] Difference between diets significant by t test of paired differences: b, $P < 0.01$; c, $P < 0.05$.

in the baboon than in the rhesus and squirrel monkeys.

The half-time for decay of specific activity of serum [^{14}C]cholesterol in animals fed the two diets is given in the bottom line of Table 5. The mean half-time was similar for baboon and rhesus monkey. The half-time for the squirrel monkey, however, was less than half of that for the baboon or rhesus monkey. There was a statistically significant decrease in mean half-time in squirrel monkeys and baboons when these animals were changed from the basal to the test diet. The increase in mean half-time for rhesus monkey was not statistically significant.

DISCUSSION

In this study we have shown that there are large differences among the baboon, the rhesus monkey, and the squirrel monkey in the average response of serum cholesterol concentration to an atherogenic diet. We have also shown that among these species the percentage of dietary cholesterol absorbed parallels the serum cholesterol concentration while they are fed the atherogenic diet. Thus, differences in ability to absorb dietary cholesterol may be one factor determining the level of serum cholesterol when these species are fed a high cholesterol diet.

In this study the increase in total rate of fecal excretion of cholesterol and its metabolites and the increase in excretion of bile acids were greatest in the rhesus monkey. This species also had the greatest increase in serum cholesterol concentration. It would seem unlikely, therefore, that differences among species in the ability to increase excretion or catabolism of cholesterol is a major factor in determining differences in response of serum cholesterol to the atherogenic diet.

The similarities and differences between cholesterol metabolism in each of the three species in this study and cholesterol metabolism in man is of interest since the ultimate goal of experimental studies using these species is to understand human disease. In making comparisons with other published data, however, it must be remembered that the diets used in this study were selected to be representative of those used in studies of experimental atherosclerosis and are generally more extreme with respect to lipid content than those encountered in human experience or those used in studies of cholesterol metabolism in man.

In humans, changes in serum cholesterol with changes in level or type of dietary fat or level of dietary cholesterol are generally less than 100 mg/dl. Therefore, this study would indicate that the response of serum cholesterol concentration of the baboon or squirrel monkey is more like that of the human than is the more extreme response of most rhesus monkeys.

In a total of 16 healthy adult men and women studied by four investigators (4, 7, 10, 11), the half-times of decay of serum specific activity ranged from 41 to 75 days, with a mean of 58 days. These values are similar to the range of half-times observed here for the baboon (Table 5) and are only slightly longer than the half-times observed in the rhesus monkeys. The squirrel monkey, however, appears to turn over its exchangeable body pool several times faster than the rhesus monkey, baboon, or human.

The fraction of the exchangeable cholesterol pool derived from diet has been reported to range from 10 to 41% for normal adult humans on a variety of diets (1, 12–14). This is considerably less than the values obtained here for baboon, rhesus monkey, and squirrel monkey (Table 4) and indicates that when high cholesterol diets are fed, the diet contributes a smaller share to the exchangeable cholesterol pool in man than it does in baboon, rhesus monkey, or squirrel monkey.

Grundy and associates (1, 15–18) have reported that in nine patients ingesting a liquid formula diet containing 40% of total calories as butter oil and 285–682 mg of cholesterol/day, the fraction of dietary cholesterol absorbed ranged from 30 to 81%, with a mean of 49%. This mean value is similar to that observed in the three species studied here; however, the wide range of values reported for man precludes meaningful comparisons with the individual species.

In the present study the cage sizes were such that the relative amount of physical activity was probably greatest in the squirrel monkeys and least in the baboons, with the rhesus monkey at an intermediate level. It may be that some of the differences in cholesterol metabolism observed here, particularly the large difference between squirrel monkey and rhesus monkey or baboon in half-time for turnover, may be due to this difference in physical activity. The cage sizes used here, however, are typical of those used in most studies of atherosclerosis, so that physical activity is one factor in which these models differ.

During the atherogenic diet period, the total rate of endogenous fecal excretion of cholesterol and its metabolites in the rhesus monkey was less than the rate of absorption of dietary cholesterol. Since endogenous synthesis must also have occurred, the total input to the cholesterol pool probably exceeded the loss in feces by somewhat more than 43 mg/day in the rhesus monkey. In the baboon, the sum of absorption and synthesis of cholesterol is also likely to have exceeded the measured rate of endogenous fecal excretion. Bhattacharyya, Connor, and Spector (19) have shown that loss of cholesterol from the skin can be appreciable in the human, and it is probable that loss through the skin also occurred in the primates in this study. This would account for some of the difference between total input and fecal excretion of cholesterol that we observed.

It has been shown that, when fed high cholesterol diets, the rhesus monkey can develop xanthoma (20) and that

there is an increase in cholesterol content of many other tissues (21). Also, during the atherogenic diet period the rhesus monkeys in this study were growing at a mean rate of 4 g/day. With an approximate exchangeable cholesterol content of 2 mg/g of body weight (21), this rate of growth would require 8 mg of cholesterol/day. It is therefore probable that in the rhesus monkey an appreciable fraction of the difference between total cholesterol input and fecal excretion is a result of increasing cholesterol pool size.

The possibility of degradation of sterols in the intestines (8, 22) must also be considered as an explanation for this apparent failure to achieve steady state. In this study both the rate of cholesterol ingestion and cholesterol absorption are calculated from the measured rate of excretion of β-sitosterol in the feces. If intestinal degradation had occurred it would cause erroneously low values for β-sitosterol excretion (8) and therefore also for both cholesterol absorption and excretion. Thus, its effect on cholesterol balance would be reduced. Furthermore, it has been shown that for the human the addition of cellulose to the diet greatly reduces the degradation of cholesterol that occurs in some patients ingesting liquid formula diets (22). Since the diets used in the present studies are based on natural grains and thus contain considerable cellulose, it is unlikely that appreciable degradation of sterol has occurred.

Because of the apparent failure to achieve a steady state, the data on excretion rates observed here cannot be assumed to represent turnover rates and must therefore be interpreted with caution. The results on percentage of dietary cholesterol absorbed, dietary contribution to cholesterol pool, and relative rates of excretion of bile acid and neutral sterols that have been discussed above should, however, not be affected appreciably. We conclude that the effect of an atherogenic diet on several parameters of cholesterol metabolism varies among the baboon, the rhesus monkey, and the squirrel monkey. In addition to the well-known difference in response of serum lipids, these species differ in the fractional rate of turnover of the exchangeable body pool of cholesterol, in the fraction of dietary cholesterol absorbed, in the contribution of bile acid and neutral sterol to the total fecal excretion of cholesterol, and in the changes in excretion of cholesterol when fed an atherogenic diet. A comparison of these data with data that have been reported for man suggests that among these three species the baboon most closely resembles man with respect to cholesterol metabolism. The differences that we have observed among species should be considered when choosing experimental animals for studies of the role of dietary factors in atherosclerosis or cholesterol metabolism.

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