Nutritional effects on blood lipid and HDL cholesterol concentrations in two subspecies of African green monkeys *(Cercopithecus aethiops)*

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Abstract The African green monkey has previously been found to be a promising model for the study of atherosclerosis. We have compared the plasma and HDL cholesterol response to dietary manipulation in the two subspecies of African green monkeys (vervets and grivets) most often imported for biomedical research purposes. Twenty vervets and 20 grivets were fed, in succession, diets containing safflower oil, butter, or lard as the principal dietary fat at a level of 40% of calories. Ten animals of each subspecies were fed the diets without added cholesterol (control groups) and 10 were fed diets with either added crystalline cholesterol (safflower oil and butter diets) or egg yolk (lard diet) to raise the diet cholesterol level at least five-fold. The effect of the type of dietary fat was that total plasma cholesterol (TPC) and HDL cholesterol concentrations were lowest while the safflower oil diet was fed, were significantly higher when butter fat diets were fed, and were highest when the egg yolk-lard based diets were fed. In addition, a significant effect of the elevated level of dietary cholesterol, independent of the type of dietary fat, was seen: a statistically significant negative correlation between TPC and HDL cholesterol concentrations was induced. In contrast, a positive correlation between TPC and HDL cholesterol concentrations was found at the lower dietary cholesterol level. Thus, the different factors (type of fat versus cholesterol) influenced lipoprotein metabolism in distinct yet related ways. Although average values for both plasma and HDL cholesterol concentrations were significantly higher in the grivet subspecies than in the vervet subspecies, the data for both subspecies fit the same regression lines. This outcome suggested that the subspecies differed in the magnitude of response rather than in the mechanism of response. —**Rudel, L. L., J. A. Reynolds, and B. C. Bullock.** Nutritional effects on blood lipid and HDL cholesterol concentrations in two subspecies of African green monkeys (*Cercopithecus aethiops*). *J. Lipid Res.* 1981. **22:** 278–286.

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The prevalence of premature deaths from cardiovascular disease in people of North America and other developed countries has stimulated the study of atherosclerosis and its etiology. The development of human atherosclerosis is influenced by both genetic and environmental factors. Among the latter, the type of dietary fat and the level of dietary cholesterol have been repeatedly implicated as important factors (1). These dietary factors influence the levels of plasma cholesterol and lipoproteins which may modify atherosclerosis (2). However, cause and effect relationships have not been established, owing in large part to the difficulty of systematic study of diet-induced atherosclerosis in human beings. The chronic nature of atherosclerosis, ethical considerations, and the difficulty of maintaining strict control of environmental variables including diet limit experimental studies in human beings.

These considerations have stimulated the search for suitable and phylogenetically related animal models in order to examine the factors affecting atherosclerosis. Nonhuman primates, including rhesus monkeys (3) and cynomolgus monkeys (4) have been used by many investigators to study experimentally induced atherosclerosis. In general, responsiveness to dietary cholesterol in these species is much exaggerated compared to that in man. One subspecies of African green monkey, the vervet, has recently been recognized in our laboratories as a good model for the study of diet-induced atherosclerosis (5). The distribution and morphology of the induced lesions in vervets were found to be similar to those seen in humans (5). The degree of responsiveness to dietary cholesterol is more like that of human beings and the plasma lipoprotein response in vervets to elevated levels of dietary cholesterol was similar to the lipoprotein distribution seen in people with heterozygous familial hypercholesterolemia (6).

In order to further evaluate the African green mon-

Abbreviations: HDL, high density lipoprotein; TPC, total plasma cholesterol.

key as an experimental model for diet-induced atherosclerosis research, we have studied the effect of varied levels of dietary cholesterol and different dietary fats on plasma cholesterol, triglyceride, and HDL cholesterol concentrations. As we were aware that some shipments of African green monkeys from commercial suppliers contained animals of the grivet subspecies (*Cercopithecus aethiops aethiops*) in addition to vervets (*Cercopithecus aethiops pygerythrus*), we also looked for subspecies differences. The majority of the study was carried out using a diet with egg yolk as the source of cholesterol and with fat as 40% of calories to mimic the typical North American diet. We also fed diets containing 40% of calories either as saturated fat (butter) or unsaturated fat (safflower oil), with or without added crystalline cholesterol.

MATERIALS AND METHODS

Animal colony

Forty (20 vervets, 20 grivets) adult male African green monkeys were purchased from an animal importer (Primate Imports, Port Washington, NY). Individuals within each subspecies were housed together with five animals per cage. Prior to start of the study all animals were quarantined for 90 days until five negative biweekly tuberculin tests had been done. The animals were then studied for a total of 25 months. Once each month, hematocrit, total serum protein, blood cell count, differential white cell count, and body weight were measured. The animals remained in good health throughout the study, and maintained constant to slightly increased body weights.

Subspecies identification

The grivet monkeys were differentiated from vervets primarily by their facial appearance. They have long white facial hair which sweeps upward and backward to form a prominent beard, and the hair on the crown of the head forms a rounded cap. In contrast, the vervet monkeys have no upward sweeping facial hair, and the hair on the crown gives their head the appearance of being flat on top. Grivets are generally the larger subspecies. The average body weight for the adult male grivets were 5.7 kg with a range of 4.2–6.7 and for the vervets was 4.8 kg with a range of 3.1–6.6. Four vervets and two grivets were karyotyped and determined to have identical diploid chromosome numbers of 60.

Diets

The diet fed for the majority (20 months) of the study was termed the 75-5 diet, and the composition of this diet is shown in **Table 1.** For 2 months of the study, the animals were fed the diet designated 75-8S that contained 40% of calories as safflower oil, **Table 2.** For 2 months of the study, the diet designated 75-8B diet was fed; it contained 40% of calories as butter fat, Table 2. For each of the diets, two levels of cholesterol were fed while the remaining ingredients were identical. Two groups (one of vervets, one of grivets) were fed diets designated control, which contained low levels of cholesterol (0.03 mg/*K*cal, 75-5; 0.16 mg/*K*cal, 75-8). Two groups were fed diets designated

TABLE 1. 75-5 Diet composition[a]

Ingredients	Control	Test
	g/100 g dry weight	
Casein	7.3	7.3
Lactalbumin	7.3	7.3
Wheat flour	31.8	31.8
Dextrin	5.5	5.5
Sucrose	4.5	4.5
Applesauce	4.1	4.1
Lard	10.9	10.9
Hegsted salts mixture	3.6	3.6
Alphacel	9.1	9.1
Complete vitamin mixture	2.3	2.3
Dried egg yolk		13.6
Egg yolk replacement[b]	13.6	

[a] Each diet contained a calorie distribution of 20:42:38 in percent as protein, lipid, and carbohydrate, respectively. The control diet contained 0.03 and the test diet 0.74 mg cholesterol/kcal.

[b] Made up to match the composition of dried egg yolk, containing in g/100 g: casein, 22; lard, 50; soybean lecithin, 16; sucrose, 2.5; Hegsted salts mixture, 1.6; and water, 7.9.

TABLE 2. 75-8 Diet composition[a]

Ingredients	75-8B		75-8S	
	Control	Test	Control	Test
	g/100 g dry weight			
Casein	9.0	9.0	9.0	9.0
Lactalbumin	8.0	8.0	8.0	8.0
Wheat flour	33.5	33.5	33.5	33.5
Dextrin	10.0	10.0	10.0	10.0
Sucrose	3.6	3.6	3.6	3.6
Applesauce	1.0	0.7	1.0	0.7
Hegsted salts mixture	3.8	3.8	3.8	3.8
Alphacel	5.0	5.0	9.5	9.5
Complete vitamin mixture	2.6	2.6	2.6	2.6
Butter	23.5	23.5		
Safflower oil			19.0	19.0
Cholesterol		0.27	0.07	0.34
β-Sitosterol	0.06	0.06		

[a] Each diet contained a calorie distribution of 19:40:41 in percent of protein, fat, and carbohydrate, respectively. The control diets contained 0.16 and the test diets 0.78 mg cholesterol/kcal. β-Sitosterol was added to the butter diets to make the butter diets equivalent in the amount of plant sterol to the safflower oil diets; cholesterol was added to the safflower control diet to make the level equivalent to the amount in the butter control diet.

TABLE 3. Fatty acid compositions[a] of diets

Fatty acid	75-5	75-8B	75-8S
		weight percent	
14:0	1.0	11.8	0.1
16:0	26.0	36.5	6.7
16:1	2.7	1.5	
18:0	12.1	12.1	2.7
18:1	45.2	26.1	12.2
18:2	12.0	2.0	78.0
18:3	0.8		0.3
Others	0.2	10.0	

[a] Diets were extracted in chloroform–methanol 2:1 and the lipid extract was dried, the residue was saponified at 80°C in alcoholic KOH for 1 hr, and the nonsaponifiable material was removed with hexane. The fatty acids were then extracted, methylated with borontrifluoride and the methyl esters were separated by GLC (16). Each set of values represents the mean of triplicate analyses, for both control and test diets. No differences in control versus test diet patterns were found.

test, which contained higher cholesterol levels (0.74 mg/*K*cal, 75-5; 0.78 mg/*K*cal, 75-8).

Dried egg yolk was the source of cholesterol in the 75-5 test diet. An egg yolk replacement mixture was used in place of the egg yolk in the 75-5 control diet. This mixture was made up to mimic egg yolk and yet be essentially cholesterol-free. The composition of the replacement was based on our analyses of the dried egg yolk used in these diets. Soybean lecithin (Central Soya Co., Ft. Wayne, IN.) was used as the phospholipid source of the egg yolk replacement mixture. We were unable to find a commercial source of cholesterol-free egg yolk lecithin. Although it had a different fatty acid composition than that for egg yolk lecithin, the final fatty acid compositions of the 75-5 control and test diets were measured and found to be similar as shown in **Table 3.** The final concentration of phospholipid in the diet, measured as phospholipid phosphorus (7), was 2.4%.

Blood samples

The animals were trained to enter a capture cage for sample collection. After a 24-hr fasting period, they were immobilized with Ketamine-HCl®, 10 mg/kg, and blood drawn from the femoral vein was placed in tubes containing EDTA, 1 mg/ml final concentration. Plasma was promptly isolated, and was maintained at 4°C until analyzed usually on the day after sampling. Agarose electrophoresis (8) was run on each sample to identify samples with chylomicra present and to screen for other abnormalities. Cholesterol and triglyceride concentrations and HDL cholesterol concentrations were measured in our Lipid Analytic Laboratory according to the Lipid Research Clinics Manual (9), using the Technicon AA-II® for cholesterol and triglyceride measurement (10). Our Lipid Analytic Laboratory is in complete compliance with the Cooperative Lipid Standardization Program of the U.S. Department of Health and Human Services.

The applicability of using the heparin-manganese method (11) for HDL-cholesterol determination in plasma samples from this species was established; the cholesterol levels of the d 1.063 g/ml infranatants obtained after ultracentrifugation in a discontinuous gradient in the SW-40 rotor (Beckman Instruments, Fullerton, CA) (12) were in agreement with the HDL cholesterol values obtained in supernatants after precipitation with heparin—1 M $MnCl_2$. An absence of β-lipoprotein in infranatants that had been concentrated five-fold was documented by agarose electrophoresis (8) demonstrating that LDL had been effectively removed. Twice during the 75-5 diet period HDL levels were determined using our combined ultracentrifugation, agarose column method (12). Excellent agreement between HDL cholesterol values obtained in all 40 animals by this method versus the heparin-manganese method was found ($r = 0.9$, $P < 0.00001$), indicating the validity of results obtained using the latter technique. Furthermore, no differences in the apolipoprotein patterns of HDL, as seen by analytical isoelectric focusing (13), were found for HDL isolated from the d 1.063 g/ml infranatant compared to HDL isolated from the heparin-manganese supernatant.[1] ApoE has not been detected in plasma HDL of normal or hypercholesterolemic African green monkeys by SDS polyacrylamide gel electrophoresis (13), thus results from the two methods would not be expected to be different due to the presence of high concentrations of the apoE-rich HDL_c, a lipoprotein fraction described by Mahley, et al. (14).

Statistical analyses

Data in the present study were analyzed using correlation and regression analysis, two-way analysis of variance, and the paired *t*-test, essentially as described by Snedecor and Cochran (15).

RESULTS

The twenty grivets and twenty vervets of the study were initially fed the 75-5 test diet for a 28-day period (**Fig. 1**). Blood samples were then collected from each animal and total plasma cholesterol concentrations (TPC) were measured. For each subspecies, two groups (control and test) of ten animals each were

[1] Parks, J. S., and L. L. Rudel. Unpublished results.

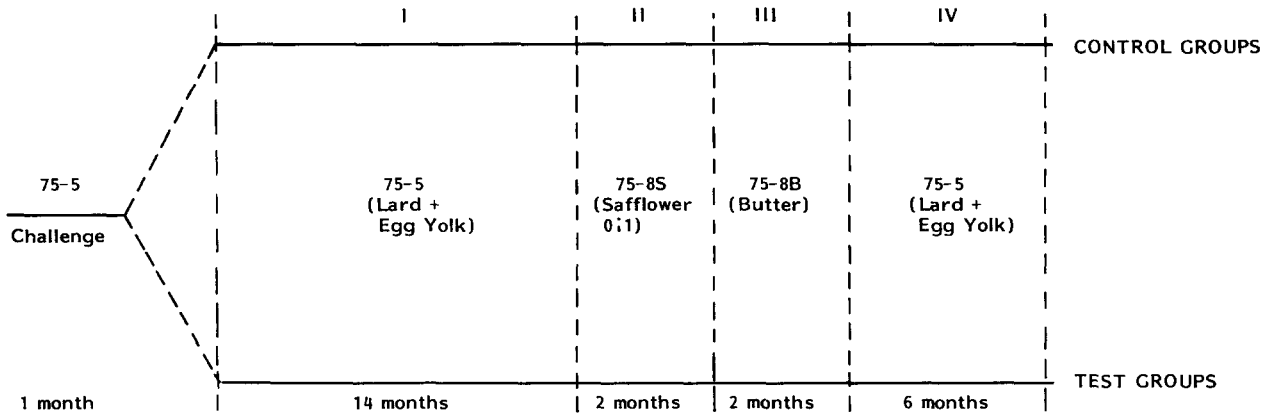

Fig. 1. Diet flow chart showing the time periods in sequence for each diet of the experiment. The diet periods are identified by a Roman numeral at the top of the figure.

selected based on the conscious bias that, within each subspecies, both groups have equivalent plasma cholesterol levels (mean ± S.D.) after the dietary cholesterol challenge. For the two grivet groups, values were 371 ± 126 and 371 ± 101 mg/dl, and for the vervet groups, 331 ± 112 and 329 ± 105 mg/dl, for animals assigned to control and test groups, respectively. Thereafter, animals were fed the appropriate 75-5 diets for 14 months. Then, all animals were fed the 75-8S safflower oil-based diets (control and test for each of these groups, respectively) for 2 months after which the 75-8B butter fat-based diets were fed for 2 months. Finally, the groups were fed the 75-5 diets for the remaining 6 months of the study. Throughout the study, the body weights (mean ± S.D.) were comparable for diet groups of vervets (4.7 ± 1.1, 4.8 ± 1.0 kg) and of grivets (5.6 ± 0.8, 5.7 ± 0.8 kg) for control and test, respectively.

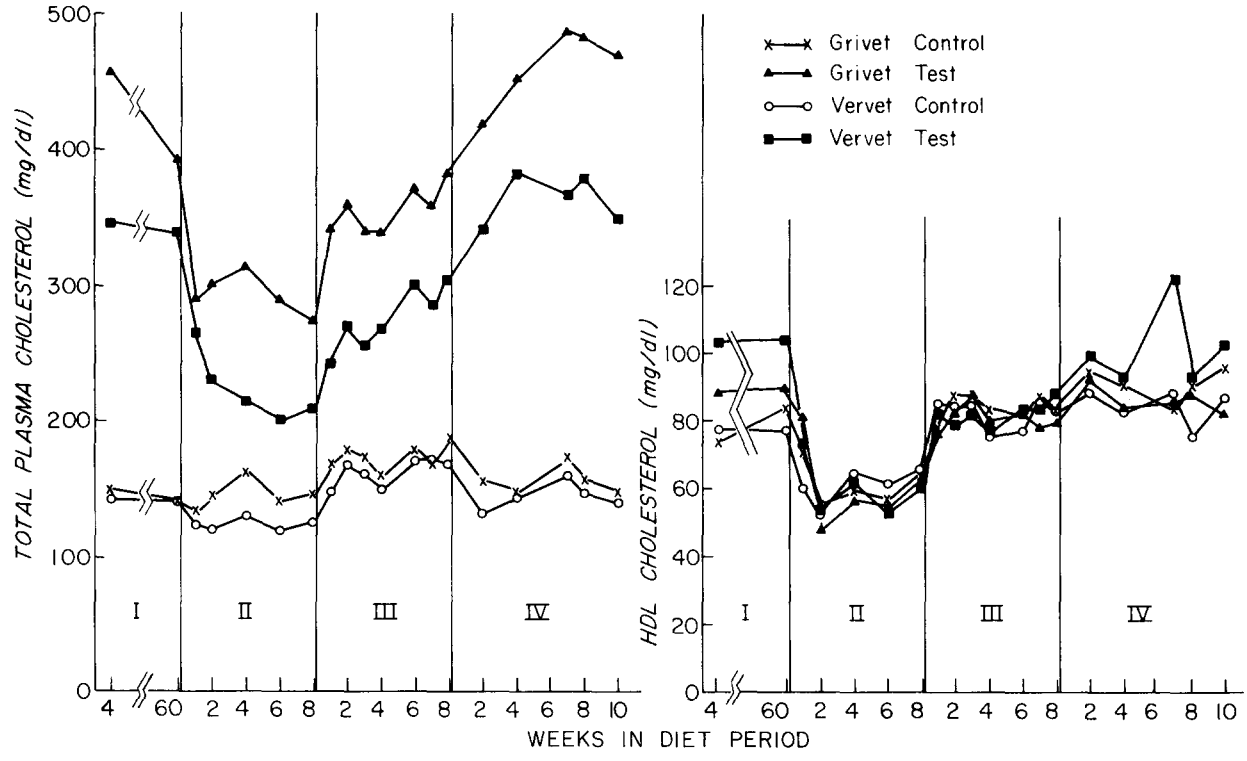

Fig. 2. Time-related changes in total plasma and HDL cholesterol concentrations when the diets for the experimental groups were changed. The Roman numerals indicate the time and diet periods as defined in Fig. 1. Each data point represents the mean value for all animals of the group. Fifteen values were taken in period I but for conciseness only representative values are shown. Likewise, only part of the data for period IV are shown.

TABLE 4. Effects of dietary fat and cholesterol on plasma and HDL cholesterol concentrations in vervets versus grivets

Subspecies	Group	Cholesterol Concentration 75-5[a]	75-8S[c]	75-8B[c]
			mg/dl	
			Whole Plasma	
Vervet	Control	142 ± 7[b]	122 ± 7	162 ± 10
Vervet	Test	344 ± 46	224 ± 40	275 ± 45
Grivet	Control	152 ± 5	145 ± 6	174 ± 8
Grivet	Test	433 ± 34	294 ± 27	356 ± 44
			HDL	
Vervet	Control	84 ± 4	60 ± 3	83 ± 4
Vervet	Test	98 ± 6	60 ± 5	82 ± 5
Grivet	Control	90 ± 5	63 ± 3	84 ± 3
Grivet	Test	87 ± 9	60 ± 5	81 ± 8

[a] Represents mean values for the 27 observations on each of the animals during the 20 months on this diet.
[b] All values, mean ± SEM for ten animals per group.
[c] Data for each animal were measured on five to eight weekly samples drawn during consecutive diet periods of 8 weeks each.

Total plasma cholesterol concentrations

Fig. 2 shows the time-related changes in total plasma cholesterol concentrations associated with the diet changes. Generally, the degree of change that occurred was greatest during the first 2 to 4 weeks of the diet period. The animals of the test groups experienced a dramatic decrease in plasma cholesterol concentration during this time that continued throughout the 8 weeks of the 75-8S polyunsaturated fat-rich test diet (period II). The values rose on the 75-8B saturated fat test diet (period III), and reached the highest values when the groups returned to the 75-5 egg yolk-lard test diet (period IV). The values during this latter period appeared to overshoot the levels of period I but then return to the earlier level. For control groups, the changes in plasma cholesterol concentrations were smaller and appeared to have occurred by 2 weeks into the diet period.

Table 4 shows the mean values (±SEM) for plasma cholesterol concentrations in each of the groups and those for all observations collected during each diet period. These mean values accurately reflect the trends shown in Fig. 2. Values for the 75-5 diets of periods I and IV have been averaged together since no statistically significant difference was found between the two periods. On all diets the vervets had lower mean plasma cholesterol concentrations than the grivets. By two way analysis of variance, this subspecies difference was found for both control diet groups, $P < 0.05$, and test diet groups, $P < 0.01$. When the animals were fed the safflower oil-based diets, they had significantly lower TPC values than when fed the butter fat-based diets, ($P < 0.001$, paired t-test). The TPC values when the animals were fed the 75-8B test diet were significantly lower ($P < 0.001$, paired t-test) than when the animals were fed the egg yolk based 75-5 test diet, even though dietary cholesterol levels were comparable. When animals were fed the 75-8B control diet, TPC values were higher ($P < 0.001$, paired t-test) than when they were fed the 75-5 control diet. This may have been due to the higher cholesterol content, 0.16 mg/kcal versus 0.03 mg/kcal for 75-8B versus 75-5 control diets, respectively.

Since each of the animals in the study was fed all three study diets for its group, it was possible to compare the individual animals for their position in the group based on TPC. Using data for the TPC of individual animals, the correlation coefficients were all above $r = 0.9$, $P < 0.001$, when comparing any two test diets, showing that rank order among the animals was constant and independent of diet. Animals that were high responders on one test diet were high on the other two, and the same was true all along the scale of response. Interestingly, the rank order among animals receiving the control diets also remained relatively constant and the correlation coefficients were also above $r = 0.9$ when comparing different control diets. In contrast, when the initial 28-day response data were used, the rank order on the test diet was not found to be predictive of the rank order on the control diet, $r = 0.4$, N.S.

High density lipoprotein cholesterol concentrations

The time- and diet-related changes in HDL cholesterol concentrations are also shown in Fig. 2. In general, the average HDL responses among diet and species groups were comparable. HDL cholesterol values decreased at the start of feeding the 75-8S polyunsaturated fat diet (period II) and remained at comparable low levels after 2 weeks into this diet period. The HDL cholesterol concentrations increased promptly after initiation of the 75-8B saturated fat diet (period III) and remained at comparable levels after 1 week on this diet. Smaller increases occurred for most groups upon return to the 75-5 diets.

The data of Table 4 include the mean values for HDL cholesterol concentrations among the different study groups. These mean values accurately reflect the trends in the time-related changes as shown in Fig. 2. HDL cholesterol concentrations were significantly lower ($P < 0.001$, paired t-test) when the animals were fed 75-8S diets versus 75-8B diets, and were significantly lower ($P < 0.01$, paired t-test) on the 75-8B diets versus the 75-5 diets. No significant subspecies

difference was apparent on any diet, and no effect of dietary cholesterol on HDL cholesterol concentration was apparent, based on an examination by two-way analysis of variance. However, this apparent lack of an effect of dietary cholesterol was not consistent with our impressions obtained during data collection, so further analyses were carried out.

The plots of the data in **Fig. 3** show the relationships between the TPC and HDL cholesterol concentration. In the case of animals fed both the control 75-8B and 75-5 diets, highly significant positive correlations existed between HDL cholesterol and TPC. The relationship was not the same for both diets, however. These two regression lines were found to be significantly different ($P < 0.01$) indicating that the relationships between TPC and HDL cholesterol were related to diet composition. Given a plasma cholesterol concentration of 150 mg/dl, the corresponding HDL cholesterol concentration based on the regression lines was 72.5 versus 88 mg/dl for the 75-8B versus the 75-5 control diets, respectively. No statistically significant correlation existed between TPC and HDL cholesterol when the 75-8S control diet was fed.

When the test diets containing the higher cholesterol levels were fed, statistically significant *negative* correlations were found between TPC and HDL cholesterol for each of the diets, in marked contrast to the statistically significant *positive* correlations found for two of the control diets. The regression lines found for the 75-5 versus the 75-8B test diets, respectively, were significantly different from each other ($P < 0.001$). As was the case for the control diet comparisons for these two diets, HDL levels were significantly higher (at equivalent TPC values) when the animals were fed the 75-5 versus 75-8B test diet. A statistically significant negative correlation was found for the TPC-HDL cholesterol relationship in the animals fed the 75-8S diet, and the regression line was significantly different from that of the 75-8B test diet ($P < 0.001$) and the 75-5 test diet ($P < 0.001$). At equivalent plasma cholesterol concentrations, HDL cholesterol levels were lower on this diet than for either of the other two diets. No significant subspecies differences between grivets and vervets were apparent from any of the plots, i.e., data for both species fit the same line within each diet situation.

Another way to view the relationship between TPC and HDL cholesterol was to examine the TPC/HDL cholesterol ratio (**Table 5**). The increase in dietary cholesterol level resulted in an increased TPC/HDL cholesterol ratio and the increase occurred on all three diets studied. No significant differences were seen in the ratio among any of the test diets. The grivet

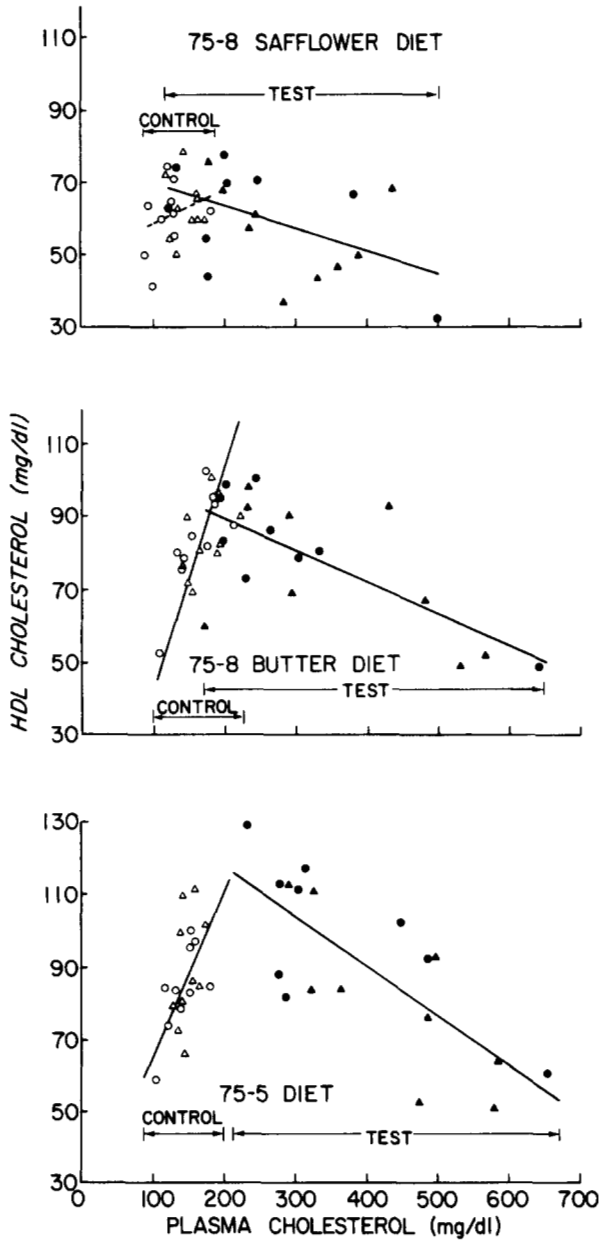

Fig. 3. Comparison of the effects of dietary fat and cholesterol on the relationships between plasma and HDL cholesterol concentrations. The diets are described in Tables 1 and 2. Each point represents the averaged data for one animal; the numbers of observations per animal are as given in Table 4. Open symbols are used for animals fed the control diets, closed symbols are used for animals fed the test diets. Equal numbers of grivets (▲, △) and vervets (●, ○) were studied on each diet and each animal was studied on each of the three diets for his group. Between plasma and HDL cholesterol concentrations, the correlation coefficients and statistical significance for each diet were: 75-8S control, $r = 0.28$, $P < 0.19$; 75-8S test, $r = -0.49$, $P < 0.04$; 75-8B control, $r = 0.65$, $P < 0.003$; 75-8B test, $r = -0.70$, $P < 0.002$; 75-5 control, $r = 0.64$, $P < 0.003$; 75-5 test, $r = -0.76$, $P < 0.001$. The least squares best fit regression line is drawn where a statistically significant correlation was found. The range for plasma cholesterol concentration values for each of the diet groups is indicated with arrows. Considerable overlap between control and test groups occurred for the 75-8S diets, however no overlap occurred for the 75-5 diet groups.

TABLE 5. Comparison among diets of the ratio of total plasma cholesterol/HDL cholesterol

Subspecies	Group	Ratio		
		75-5	75-8S	75-8B
Vervet	Control	1.70 ± 0.07[a]	2.04 ± 0.14	1.95 ± 0.08
Vervet	Test	3.88 ± 0.85	4.33 ± 1.31	3.82 ± 1.07
Grivet	Control	1.72 ± 0.08	2.33 ± 0.12	2.08 ± 0.09
Grivet	Test	5.77 ± 0.98	5.34 ± 0.72	5.09 ± 1.06

[a] All values, mean ± SEM for at least five observations on each of ten animals per group.

subspecies responded to the higher dietary cholesterol level with a greater increase in the ratio. The TPC/HDL cholesterol ratio was significantly ($P < 0.05$) higher for grivets than for vervets on each of the test diets, but no significant difference between subspecies was seen on the control diets.

Triglyceride concentrations

The plasma triglyceride concentrations are shown in **Table 6.** The values are uniformly low and are comparable between groups. No significant dietary effects were found for any of the groups.

DISCUSSION

A high degree of constancy was found when the animals in this study were rank-ordered in terms of their TPC. The unsaturated fat of the 75-8S diet lowered the TPC values, but they were lowered in each animal so that the rank order among animals on control and test diets remained the same as was found during feeding of the 75-8B diet and the 75-5 diet. This pattern of constancy in the rank order among diets was also found in the HDL cholesterol values. These individual animal differences are important because they were used to illustrate the effects of dietary constituents on plasma lipoproteins as shown in Fig. 3. The relationships between HDL and total plasma cholesterol concentrations are described by the regression lines of Fig. 3. A positive correlation between these values was found when control diets containing saturated fat were fed. This relationship became a negative correlation when the dietary cholesterol level was increased. These data show that dietary cholesterol level influences HDL cholesterol level differently among individuals but in a definite pattern. Animals generally responded to dietary cholesterol with an increase in TPC, while HDL cholesterol concentrations decreased in proportion to this increase. When the relationship between HDL and TPC was examined for the different test diets, a statistically significant difference was found between the regression lines; in animals with the more modest plasma cholesterol responses, HDL cholesterol concentrations from highest to lowest were: 75-5 test > 75-8B test > 75-8S test. Thus, in the individuals with low to moderate responses to dietary cholesterol, the HDL cholesterol levels were dependent on the type of fat in the diet and possibly on the lecithin in the egg yolk diet. In the most responsive animals, the HDL cholesterol levels were lowest and essentially equivalent on each diet. A dietary fat effect in addition to the cholesterol effect was not evident in these cases. It is important to emphasize the necessity of using regression analyses and individual animal variability to find most of these nutritional effects on HDL cholesterol concentration. Many of the differences are not apparent based on mean (±SEM) values (Table 4).

These effects of dietary cholesterol and of the dietary fat on HDL levels have not been described previously by others. We have shown that the dietary cholesterol effect holds for total HDL concentration versus plasma cholesterol concentration in African green monkeys (17). We have previously described the different HDL responses to dietary cholesterol that occur in rhesus monkeys (18), namely that HDL cholesterol decreased in animals that achieved high plasma cholesterol concentrations and increased in those animals that reached modest plasma cholesterol elevations. The present data in African green monkeys are consistent with these earlier observations, and in fact, this relationship is generally true across species of nonhuman primates (17). In addition, the polyunsaturated fat-induced lowering of total plasma and HDL cholesterol concentrations reported here in nonhuman primates is similar to that reported by Shepherd, et al (19) in studies of human primates.

The fact that a positive linear relationship between

TABLE 6

Comparison of plasma triglyceride concentrations in subspecies of African green monkeys fed different diets

Subspecies	Group	N	Plasma Triglyceride		
			75-5[a]	75-8S[b]	75-8B[b]
			mg/dl		
Vervet	Control	10	20 ± 2	21 ± 3	36 ± 6
Vervet	Test	10	21 ± 3	29 ± 4	28 ± 4
Grivet	Control	10	17 ± 1	11 ± 2	26 ± 4
Grivet	Test	10	16 ± 3	21 ± 8	28 ± 7

[a] All values are mean ± SEM for at least 16 observations per animal.

[b] All values are mean ± SEM for two observations per animal.

HDL cholesterol concentration and total plasma cholesterol concentration becomes an inverse linear relationship in African green monkeys fed increased cholesterol levels suggests that the HDL response is an integral part of the response to dietary cholesterol. The mechanism for this response is unknown, but enough information exists to permit speculation. Many of the concepts used in our reasoning have been recently reviewed by Tall and Small (20). The fact that cholesterol feeding stimulates an increase in HDL cholesterol concentration in some animals (those showing minimal TPC responses to dietary cholesterol) suggests that the increased demand for cholesterol transport can result in increased HDL cholesterol levels. An increased amount of free cholesterol is put into the circulation during cholesterol feeding via cholesterol absorption in African green monkeys (21) and an increased synthesis of cholesteryl esters with subsequent transport in HDL could result. It is currently believed that newly formed or "nascent" HDL are discs of phospholipid, apolipoprotein, and free cholesterol and are deficient in cholesteryl esters. An increase in the amount of cholesteryl ester in HDL arising via increased ester synthesis presumably would occur due to intravascular LCAT activity (22). Since apoA-I and apoA-II are found on chylomicra derived from the intestine of African green monkeys (23), and the apoA-I, apoA-II, and phospholipid of chylomicra have been shown to transfer to the HDL fraction during catabolism (23–25), it seems likely that one factor involved in elevating HDL concentrations would be the increased availability of HDL precursors from the surface of intestinally derived lipoprotein particles, including the free cholesterol transported from the gut during cholesterol feeding.

In contrast, animals that were maximally responsive to dietary cholesterol had decreased HDL levels. Presumably this could be due to increased transport of diet-derived cholesteryl ester in lymph chylomicra and VLDL, since in animals known to be hyperresponsive to dietary cholesterol, such as the rabbit, dietary cholesterol is preferentially esterified (26). In this circumstance, a much larger remnant with an increased cholesteryl ester content per particle would result from chylomicron degradation. The relative amount of redundant surface material available for HDL formation (20) would be reduced in this case. A decreased HDL concentration could be a result. This hypothetical pathway is only speculative, but it does permit us to design further experiments so that the relative validity of this pathway can be tested.

When the animals were fed the polyunsaturated fat diets, lower HDL cholesterol concentrations resulted, although the inverse relationship of the HDL to plasma cholesterol concentration was still seen in the animals fed the higher cholesterol level. During the polyunsaturated fat-feeding period, HDL concentrations may have been limited by the amount of precursor materials from the surface of chylomicra. Ockner, Hughes, and Isselbacher (27) have suggested that intestinal lipoproteins formed during absorption of polyunsaturated fatty acids are larger than particles formed from more saturated fats. In African green monkeys, lymph chylomicra obtained from safflower oil-fed animals are about 400 Å in diameter larger than those obtained from butter fed animals.[1] This may be due, at least in part, to the more rapid absorption facilitated by the greater fluidity of polyunsaturated fat at body temperature. The larger particles would provide less surface material for a given amount of transported fat than would be provided by smaller particles. The relative shortage of HDL precursors (phospholipids, apoproteins A-I and A-II) derived from chylomicra surface of polyunsaturated fat-fed animals may be a reason for the lower HDL cholesterol concentrations seen in the African green monkeys.

The highest HDL levels were found when the animals were fed the 75-5 diets. The inverse relationship of HDL to plasma cholesterol concentrations was still present (Fig. 3). This finding may be related to the mechanism proposed above through the increased content of lecithin in the egg yolk-based 75-5 diet, in addition to the fact that this is a saturated fat diet. The presence of increased lecithin in the intestine during fat absorption can result in an increased availability of lecithin for forming chylomicron surface (28, 29). In this circumstance, the surface to core ratio would be higher and the average size of the particles smaller so that the amount of HDL precursors transported as chylomicron surface materials would be increased per fat meal. This is essentially the inverse of the polyunsaturated fat effect, with average particle size being a key. In this case as well as those discussed above, individual animal responsiveness to dietary cholesterol via cholesterol absorption would be evident. Lipoprotein metabolism subsequent to absorption would reflect the effects of both dietary components, i.e., cholesterol and lecithin, with the individual animal variability still being expressed. Thus, the data in the present study appear to define distinct yet interrelated plasma TPC and HDL responses to dietary cholesterol, dietary fat, and perhaps even lecithin. The separateness of the response to each component is emphasized by the fact that the further effect of dietary fat and lecithin can be superimposed on the dietary cholesterol effect with the result being a modification of the inverse

linear relationship between HDL and total cholesterol concentration.

The average total plasma cholesterol response of grivets was higher than that of vervets. However, essentially complete overlap among the individuals of each subspecies is shown in the graphs of Fig. 2 and the data for both subspecies fit the same regression line. This suggests that the mechanisms determining TPC responses to diet were similar for both subspecies but that grivets were relatively more responsive to dietary cholesterol than were vervets.

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REFERENCES

1. Ahrens, E. J., Jr., W. E. Connor, E. L. Bierman, C. J. Glueck, J. Hirsch, H. C. McGill, Jr., N. Spritz, L. Tobian, Jr., and T. B. Van Itallie. 1979. Symposium on the evidence relating six dietary factors to the nation's health. *Am. J. Clin. Nutr.* **32:** 2621–2748.
2. Stamler, J. 1979. Public health aspects of optimal serum lipid-lipoprotein levels. *Prev. Med.* **8:** 753–759.
3. Manning, P. J., and T. B. Clarkson. 1972. Development, distribution, and lipid content of diet-induced atherosclerotic lesions of rhesus monkeys. *Exp. Mol. Pathol.* **17:** 38–54.
4. Armstrong, M. L., and M. B. Megan. 1975. Arterial fibrous proteins in cynomolgus monkeys after atherogenic and regression diets. *Circ. Res.* **36:** 256–261.
5. Bullock, B. C., N. D. M. Lehner, T. B. Clarkson, M. A. Feldner, W. D. Wagner, and H. B. Lofland. 1975. Comparative primate atherosclerosis. I. Tissue cholesterol concentration and pathologic anatomy. *Exp. Mol. Pathol.* **22:** 151–175.
6. Clarkson, T. B., R. W. Prichard, B. C. Bullock, R. W. St. Clair, N. D. M. Lehner, D. C. Jones, W. D. Wagner, and L. L. Rudel. 1976. Pathogenesis of atherosclerosis: some advances from using animal models. *Exp. Mol. Pathol.* **24:** 264–286.
7. Fiske, C. H. and Y. SubbaRow. 1925. Colorimetric determination of phosphorus. *J. Biol. Chem.* **66:** 357–400.
8. Noble, R. P. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9:** 693–700.
9. Manual of Laboratory Operations Lipid Research Clinics Program. 1974. Vol 1. Lipid and Lipoprotein Analysis. DHW Publication no. (NIH) 75-628. 1–81.
10. Rush, R. L., L. Leon, and J. Turrell. 1970. Automated simultaneous cholesterol and triglyceride determination on the Auto Analyzer II. *In* Advances in Automated Analysis-Technicon International Congress. Thurman Associates, Miami, FL. 503–507.
11. Burstein, M., and J. Samaille. 1960. Sur un dosage rapide du cholesterol lie aux α-et aux β-lipoproteines du serum. *Clin. Chim. Acta.* **5:** 609.
12. Rudel, L. L., D. G. Greene, and R. Shah. 1977. Separation and characterization of plasma lipoproteins of rhesus monkeys. (*Macaca mulatta*). *J. Lipid Res.* **18:** 734–744.
13. Parks, J. S., and L. L. Rudel. 1979. Isolation and characterization of high density lipoprotein apoproteins in the nonhuman primate (vervet). *J. Biol. Chem.* **254:** 6716–6723.
14. Mahley, R. W., T. L. Innerarity, T. P. Bersot, A. Lipson, and S. Margolis. 1978. Alterations in human high density lipoproteins, with or without increased plasma cholesterol, induced by diets high in cholesterol. *Lancet.* **2:** 807–809.
15. Snedecor, G. W., and W. G. Cochran. 1971. Statistical Methods. Iowa State University Press, Ames, IA.
16. Kuksis, A. 1971. Progress in analysis of lipids IX. Gas chromatography, Part I. *Fette Seifen. Anstrichm.* **73:** 130–138.
17. Rudel, L. L. 1980. Plasma lipoproteins in atherogenesis in nonhuman primates. *In* Use of Nonhuman Primates in Cardiovascular Research. S. S. Kalter, editor. University of Texas Press, Austin and London. 37–57.
18. Rudel, L. L., and H. B. Lofland, Jr. 1976. Circulating lipoproteins in nonhuman primates. *In* Primates in Medicine, Atherosclerosis in Primates. J. P. Strong, editor. Karger, Basel. 224–266.
19. Shepherd, J., C. J. Packard, J. R. Patsch, A. M. Gotto, and O. D. Taunton. 1978. Effects of dietary polyunsaturated and saturated fat on properties of high density lipoproteins and metabolism of apolipoprotein A-I. *J. Clin. Invest.* **61:** 1582–1592.
20. Tall, A. R., and D. M. Small. 1978. Plasma high density lipoproteins. *N. Engl. J. Med.* **299:** 1232–1236.
21. Klein, R. K., and L. L. Rudel. 1979. Intestinal regulation of cholesterol transport by lymph lipoproteins during absorption. *Circulation.* **59:** II–18.
22. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin: cholesterol acyltransferase: perspectives from pathology. *Adv. Lipid Res.* **11:** 1–65.
23. Parks, J. S., and L. L. Rudel. 1979. Different kinetic fates of apolipoproteins A-I and A-II from lymph chylomicra. *Circulation.* **59:** II–32.
24. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high-density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64(1):** 162–171.
25. Tall, A. R., P. H. R. Green, R. M. Glickman, and J. W. Riley. 1979. Metabolic fate of chylomicron phospholipids and apoproteins in the rat. *J. Clin. Invest.* **64:** 977–989.
26. Rudel, L. L., M. D. Morris, and J M. Felts. 1972. The transport of exogenous cholesterol in the rabbit. I. Role of cholesterol ester of lymph chylomicron and lymph very low density lipoproteins in absorption. *J. Clin. Invest.* **51:** 2686–2692.
27. Ockner, R. K., F. B. Hughes, and K. J. Isselbacher. 1969. Very low density lipoproteins in intestinal lymph: role in triglyceride and cholesterol transport during fat absorption. *J. Clin. Invest.* **48:** 2367–2373.
28. Mansbach, C. M., II. 1977. The origin of chylomicron phosphatidylcholine in the rat. *J. Clin. Invest.* **60:** 411–420.
29. Scow, R. O., Y. Stein, and O. Stein. 1967. Incorporation of dietary lecithin and lysolecithin into lymph chylomicrons in the rat. *J. Biol. Chem.* **242:** 4919–4924.