Effects on plasma lipoproteins of monounsaturated, saturated, and polyunsaturated fatty acids in the diet of African green monkeys

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Abstract Work by other investigators has shown that an increase in dietary content of monounsaturated fatty acids can result in a decreased plasma low density lipoprotein (LDL) cholesterol concentration. This observation, combined with the epidemiologic evidence that monounsaturated fat-rich diets are associated with decreased rates of death from coronary heart disease, suggests that inclusion of increased amounts of monounsaturated fat in the diet may be beneficial. The present study was carried out in a primate model, the African green monkey, to evaluate the effects of dietary monounsaturated fat on plasma lipoprotein cholesterol endpoints. Two study periods were carried out in which the fatty acid compositions of the experimental diets were varied. All diets contained 35% of calories as fat. In the first experimental period, a mixture of fats was used to set the dietary fatty acid composition to be approximately 50–60% of the desired fatty acid, either saturated, monounsaturated, or polyunsaturated (n-6). In the second experimental period, pure fats were used (palm oil, oleic acid-rich safflower oil, and linoleic acid-rich safflower oil) to maximize the difference in fatty acid composition. The effects of the more exaggerated dietary fatty acid differences of period 2 were similar to those that have been reported in humans. For the group fed the diet enriched in monounsaturated fat compared to saturated fat, whole plasma and LDL cholesterol concentrations were significantly lower while high density lipoprotein (HDL) cholesterol concentrations were not affected. For the group fed the diet enriched in polyunsaturated fat compared to saturated fat, both LDL and HDL cholesterol concentrations were significantly lower than in the group fed saturated fat. LDL cholesterol concentrations were comparable in the monounsaturated and polyunsaturated fat groups and the percentage of cholesterol in LDL was lowest in the monounsaturated fat fed group. Trends were similar for the mixed fat diets, although no statistically significant differences in plasma lipoprotein endpoints could be attributed to monounsaturated fatty acids in this dietary comparison. Since effects on plasma lipoproteins similar to those seen in humans were identified in this primate model, relevant mechanisms for the effects of dietary fatty acids on lipoprotein endpoints related to coronary artery atherosclerosis, per se, can subsequently be examined. —**Rudel, L. L., J. L. Haines, and J. K. Sawyer.** Effects on plasma lipoproteins of monounsaturated, saturated, and polyunsaturated fatty acids in the diet of African green monkeys. *J. Lipid Res.* 1990. **31:** 1873–1882.

Supplementary key words apolipoprotein A-I • apolipoprotein B • cholesterol • high density lipoproteins • low density lipoproteins • particle size • plasma concentrations • triglycerides

The role of blood plasma cholesterol as a factor important in stimulating atherogenesis is now widely accepted. A role for nutrition in modifying plasma cholesterol concentrations is also generally recognized. Cholesterol and saturated fat in the diet increase total plasma cholesterol concentrations (1). Dietary polyunsaturated fat generally lowers plasma cholesterol, although identification of the specific effects of different polyunsaturated fatty acids is not certain. The early studies of Ahrens et al. (2) suggested that the iodine number of dietary fat was correlated to cholesterol lowering. Other work suggested that polyunsaturated fatty acids from plant sources (principally n-6 fatty acids) were about one-half as effective at lowering plasma cholesterol as saturated fatty acids were at increasing plasma cholesterol, while monounsaturated fatty acids appeared to have no effect (3, 4). These studies are among those that formed the basis for the trend over the last two decades to increase the polyunsaturated fat and to decrease the saturated fat content of our diet (5). More recently, n-3 polyunsaturated fatty acids have received increasing attention, and have been found to have some plasma cholesterol lowering effects (6), although the degree to which this effect is related to a simultaneous decrease in saturated fatty acid content is uncertain since not all studies agree (7).

The role of monounsaturated fatty acids is now being reinvestigated. The studies of Keys, Anderson, and Grande (3) and then of Hegsted et al. (4) indicated that monounsaturated fatty acids had little if any effect on plasma cholesterol concentrations. In recent years, attention has been shifted from studies on plasma 'total' cholesterol to a definition of the effects on LDL and HDL cholesterol. Different conclusions have been reached con-

Abbreviations: ANOVA, analysis of variance; ANCOVA, analysis of covariance; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; apoE, apolipoprotein E; EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoproteins; LDL, low density lipoproteins; TPC, total plasma cholesterol; VLDL, very low density lipoproteins.

cerning the role of monounsaturated fatty acids. In studies of institutionalized individuals fed liquid diets with large substitutions of monounsaturated fatty acids for either saturated or polyunsaturated fatty acids, Mattson and Grundy (8) reported that monounsaturated fatty acids are as effective as polyunsaturated fatty acids at lowering LDL cholesterol concentrations. In addition, these studies confirmed an earlier observation by Shepherd et al. (9) that large amounts of polyunsaturated fatty acids lower plasma HDL cholesterol concentrations but monounsaturated fatty acids did not have this effect (8). Other studies showing that monounsaturated fat compared to saturated fat in the diet may lead to lower LDL cholesterol concentrations have also appeared (10–12). Some investigators have found that increasing the content of fat in the diet with monounsaturated fatty acids can lead to lower plasma LDL concentrations (13, 14). Interest in the role of monounsaturated fatty acids in coronary heart disease prevention has recently been heightened by the update of the Seven Countries Study (15). After 15 years of follow-up, and after 2,288 deaths occurred in the study population, the all causes death rate was negatively correlated to monounsaturated fat intake and the coronary heart disease death rate was found to be relatively low in the cohorts in which olive oil was the main source of visible fat in the diet.

These observations indicate the importance of identification of specific mechanisms for dietary fatty acid effects. Invasive studies will be needed to define specific molecular mechanisms. In order to identify an appropriate animal model in which mechanisms can be studied, and to learn whether the effects of dietary monounsaturated fatty acids on atherosclerosis, per se, can be documented, we initiated the present studies in African green monkeys. We have previously documented the similarities with humans in the plasma lipoprotein response of this primate species to dietary polyunsaturated and saturated fat (16, 17) and have shown that approaches to definition of molecular mechanisms of dietary fat are feasible in this species (18, 19). We also have shown that effects of dietary fat on atherosclerosis outcome can be measured in African green monkeys (20). Therefore, the goal of the present study was to determine whether dietary monounsaturated fatty acids affect plasma lipoproteins in this model in a manner similar to that seen in humans. When compared to a saturated fat-enriched diet, plasma LDL cholesterol concentrations were lowered significantly by a diet rich in monounsaturated fat and by a diet rich in polyunsaturated fat; plasma HDL cholesterol concentrations were significantly lowered by the polyunsaturated fat-enriched diet but not by the monounsaturated fat-enriched diet. This outcome is essentially the same as that found in humans in the studies of Mattson and Grundy (8). After a 5 year atherosclerosis progression period, the primates of these studies will be used to determine whether there is a dietary fatty acid effect directly on coronary artery atherosclerosis.

METHODS AND MATERIALS

The animals were adult male African green monkeys (*Cercopithecus aethiops*) purchased from Primate Imports, Port Washington, NY, and from the breeding colony at Hahnemann Medical School. The animals obtained were a mixture of vervets (*C. aethiops pygerythrus*) and grivets (*C. aethiops aethiops*). Forty four animals began the study. All animals were individually caged and fed a monkey chow diet for at least 10 weeks during the initial quarantine period. Then, for the next 11 weeks of the study, the challenge period, all animals were fed an atherogenic diet that contained 0.8 mg/kcal of cholesterol and 40% of calories as saturated fat (mainly lard) (16). Three experimental groups of animals were then established, two groups of 15 animals and one group of 14 animals. Each group had equivalent means (± SEM) for several parameters determined during the challenge period. The reference parameters included: plasma concentrations of total cholesterol, very low density lipoproteins plus intermediate-sized low density lipoproteins (V+ILDL) cholesterol, low density lipoproteins (LDL) cholesterol, high density lipoproteins (HDL) cholesterol, apoB, apoA-I, apoE, and LDL particle size. In addition, equivalence among experimental groups was established for body weight, systolic and diastolic blood pressure, and fasting blood glucose. Blood lipid values for all groups during a monkey chow diet period before and after the challenge diet were also equivalent. There were no differences among grivets and vervets in any of these parameters except body weight (6.5 ± 1.6 and 5.4 ± 1.1, respectively). Nevertheless, each experimental group was assigned comparable numbers of both subspecies. This method of group selection was used to assure that the experimental groups were as representative of each other as possible in response to an atherogenic diet; measurements of many of these parameters when the animals were fed monkey chow generally did not correlate, in rank order, with the values attained during the challenge period. Three animals from the study (one from each group) died before the study was complete. All deaths resulted from pneumonia or enteric disease unrelated to the experiment.

Groups were then arbitrarily assigned to be the saturated, monounsaturated, or polyunsaturated fat group. A 6-week period of feeding the monkey chow diet followed both the challenge diet and, subsequently, the period 1 diet. Two successive experimental diet periods of about 1 year were carried out. The compositions of each of the diets are given in **Table 1.** All diets contained 35% of calories as fat. Diet period 1 was termed the mixed fat period and diet period 2, the pure fat period. The dietary

TABLE 1. Diet compositions[a]

Same for All Experiments	
Ingredient	Amount
	g/100 g
Fat*	16.4
Wheat Flour	35.0
Dextrin	9.6
Sucrose	10.0
Casein	9.0
Lactalbumin	5.0
Alphacel	7.0
Crystalline Cholesterol	0.34
Hegsted Salts Mixture	5.0
Vitamin Mixture	2.6
Vitamin D_3 in Oil	0.06

*Fat for Each Experimental Group and Diet Period (g/100 g)

Period	Saturated		Monounsaturated		Polyunsaturated	
1	Lard	15.6	Oleinate[b]	9.8	Safflower oil	9.8
1	Safflower oil	0.8	Lard	6.6	Lard	6.6
2	Palm oil	15.4	Oleinate	16.4	Safflower oil	16.4
2	Safflower oil	1.0				

[a]All diets contained a calorie distribution of 35% fat, 48% carbohydrate, and 17% protein.
[b]Oleinate is oleic acid-rich safflower oil.

fats used in period 2 were those used in the study of Mattson and Grundy (8), i.e., palm oil, oleic acid-rich safflower oil (oleinate), and linoleic acid-rich safflower oil for saturated, monounsaturated, and polyunsaturated fat, respectively. All diets contained cholesterol at a level of 0.8 mg/kcal which is sufficient to induce an average level of hypercholesterolemia of between 200 and 400 mg/dl, the range of high risk in the North American population, and a range that would permit evaluation of atherosclerosis in these animals at the end of the study, a primary goal. Measured fatty acid compositions of the diets for both periods are shown in **Table 2.** Fatty acid analyses were measured by GLC using procedures previously described (21).

Blood samples were taken periodically for routine lipoprotein and lipid analyses. All blood was drawn by venipuncture from the femoral vein of animals previously fasted overnight. Ketamine hydrochloride (10 mg/kg), administered 10 min prior to blood drawing, was used to restrain the animals during blood collection. Blood samples (10 ml) were taken at 2-week intervals for the first 8 weeks of the experimental diet period, and the frequency was then gradually decreased to once every 8–9 weeks. All blood was drawn into Vacutainer tubes containing EDTA (1 mg/ml) as an anticoagulant, and these tubes were placed on ice until plasma and cells were separated (within 3 h of venipuncture) by centrifugation for 20 min at 1,000 *g* in a refrigerated centrifuge. The antibiotic and protease mixture described by Edelstein and Scanu (22) was then added, and aliquots of plasma were taken for apolipoprotein analyses and frozen at −70°C. Apolipoprotein determinations for apoA-I, apoB, and apoE were done using an ELISA procedure, essentially as described before (18, 23). A separate aliquot of plasma was used for determination of total cholesterol and triglyceride concentrations using automated enzymatic procedures with the Technicon RA-1000. The cholesterol procedure was based on the method of Allain et al. (24) using the Boehringer Mannheim Diagnostics High-Performance Cholesterol Reagent (no. 236691) and the triglyceride procedure was based on the method of Fossati and Prencipe (25) using the Technicon GPO Blank Method (no. SM4-0189K87) and reagents. Plasma HDL cholesterol was determined after heparin-manganese precipitation using the methods described in the Manual of Laboratory Operations of the Lipid Research Clinics Program (26).

At 9 weeks into each diet period, a separate 25-ml blood sample was taken for lipoprotein separation, measurement of LDL size, and determination of cholesterol distribution (27). Briefly, the procedure was as follows. A blood

TABLE 2. Diet fatty acid compositions

	Fatty Acids						
Diet	14:0	16:0	18:0	16:1	18:1	18:2	Others
				percent (w/w)			
Saturated							
Period 1	1.5 ± 0.0	24.9 ± 0.1	15.4 ± 0.2	2.2 ± 0.0	40.2 ± 0.3	15.0 ± 0.3	0.8 ± 0.3
Period 2	0.9 ± 0.2[c]	40.8 ± 0.3[a]	4.3 ± 0.1[a]	0.01 ± 0.0[a]	37.0 ± 0.2[a]	15.2 ± 0.5	1.8 ± 0.6
Monounsaturated							
Period 1	0.3 ± 0.2	12.0 ± 1.0	6.5 ± 0.7	0.8 ± 0.1	63.0 ± 1.3	17.0 ± 0.5	0.4 ± 0.3
Period 2	0.1 ± 0.0	5.8 ± 0.1[a]	2.4 ± 0.1[a]	0.1 ± 0.0[a]	71.8 ± 0.44[a]	18.8 ± 0.3[c]	1.0 ± 0.3
Polyunsaturated							
Period 1	0.9 ± 0.1	15.3 ± 0.1	8.1 ± 0.4	1.1 ± 0.1	22.7 ± 2.2	51.9 ± 3.3	0.01 ± 0.0
Period 2	0.01 ± 0.0[a]	7.1 ± 0.1[a]	2.4 ± 0.1[a]	0.01 ± 0.0[a]	12.5 ± 0.7[b]	77.0 ± 1.0[a]	0.9 ± 0.4

All values, mean ± SEM. Five to 11 diet samples of each diet were analyzed and the differences were evaluated for statistical significance by a two-tailed unpaired *t* test. When compared to the comparable diet of period one, differences were: [a]$P < 0.0001$; [b]$P < 0.002$; [c]$P < 0.01$.

sample from each animal was drawn into a disposable 30-cc syringe and was then transferred immediately into a plastic tube containing EDTA and the protease mixture. Plasma was then separated and lipid and apolipoprotein analyses were done as above. Lipoproteins were removed from plasma by centrifugation for 24 h, 15°C, at 50,000 rpm in a Beckman Ti 70.1 rotor at a densitiy of 1.225 g/ml. Lipoprotein classes were then separated by gel filtration chromatography and the cholesterol distribution and LDL molecular weights were then measured (27). The gel filtration column used in these experiments was a 25-cm Superose 6 column (Pharmacia) which was run at 0.5 ml/min with 0.9% NaCl containing 0.1% EDTA and 0.1% sodium azide as the eluant on a DuPont Model 850 high pressure liquid chromatograph.

Statistical analyses were done using the Statview™SE+ program for the Macintosh computer and with the SPSS program on a Vax 750 computer. Where appropriate, one-way analysis of variance (ANOVA) with the Fisher's post-hoc least significant difference test, repeated measures ANOVA, analysis of covariance (ANCOVA), and paired *t* test were used. ANCOVA was used, with total plasma cholesterol (TPC) as the covariate, in evaluation of LDL and HDL cholesterol endpoints to demonstrate whether dietary fatty acid effects on these endpoints occurred independently of those on TPC. ANCOVA was used for LDL and HDL cholesterol since these are the two main components of TPC.

RESULTS

All animals were initially characterized for atherogenic diet responsiveness to permit selection of experimental groups with similar average response and variability. For this dietary challenge period, a saturated fat and cholesterol-containing diet was fed for 11 weeks. Blood samples from each animal were taken at 2-week intervals to monitor the time course of TPC and HDL cholesterol response to diet, and the data are plotted in **Fig. 1.** A plateau of responsiveness for TPC had been reached by 6 weeks and the HDL cholesterol response transiently increased, then appeared to have leveled off by 8 weeks. Therefore, a more detailed characterization of lipoprotein cholesterol distribution was done at 9 weeks. These data were used to establish three equivalent experimental groups; the means (± SEM) for each of the selected groups are shown in **Table 3.** All of the groups had equivalent means and variance for each parameter.

At the end of the challenge period, the diet for all of the animals was shifted to monkey chow for 6 weeks so that plasma cholesterol concentrations could return to initial levels. The experimental diets of period 1, containing different types of fat, were then begun and the responses of plasma cholesterol (TPC) across the 66 weeks of this period are plotted in the top panel of **Fig. 2.** There were no statistically significant differences as detected by repeated measures analysis of variance among the TPC responses of the three different groups during period 1, although the average TPC for the monounsaturated fat group remained intermediate to that of the saturated fat (highest) and polyunsaturated fat (lowest) groups throughout this period. At the end of diet period 1, which lasted 66 weeks, the animals were returned to a monkey chow diet for 6 weeks, and then the experimental diets of period 2 were begun. The TPC data for the first 48 weeks of diet period 2 are plotted in the lower panel of Fig. 2. The trend for all groups was the same as in period 1, i.e., the TPC values peaked at about 6–8 weeks and then remained on a plateau. During period 2, the TPC values for the different fat groups were significantly different ($P < 0.05$, one-way ANOVA) among groups. A statistically significant difference between saturated and polyunsaturated fat groups was maintained throughout the period, but sig-

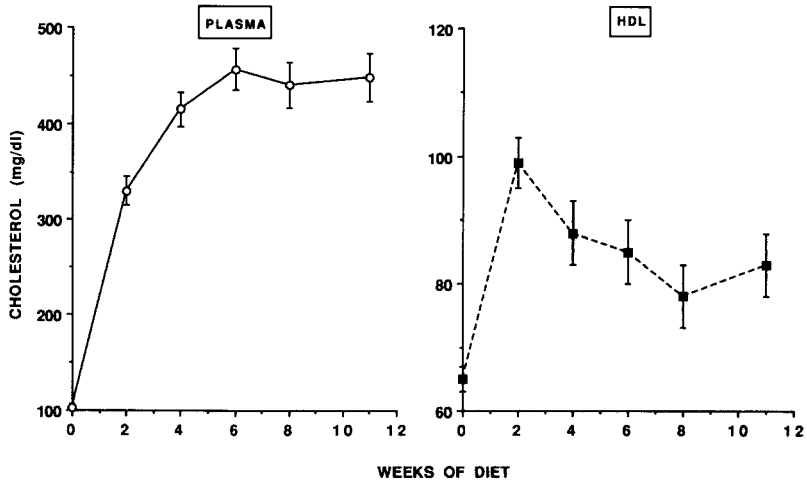

Fig. 1. Time course of TPC (left panel) and HDL cholesterol (right panel) response to an atherogenic diet in all 44 African green monkeys of the study. Each point represents the mean ± SEM. The values at zero time are taken before the diet was fed on the first day of the period. The monkeys were fed monkey chow for the preceeding 3 months.

TABLE 3. Experimental group selection based on response to an atherogenic diet

Group	Body Weight	Cholesterol				TG	ApoA-I	ApoB	ApoE	LDL MW
		Whole Plasma	V + ILDL	LDL	HDL					
	kg					mg/dl				*g/μmol*
SAT	5.8 ± 1.1	387 ± 33	38 ± 6 (9 ± 1)	273 ± 31 (68 ± 3)	78 ± 9 (23 ± 3)	19 ± 2	252 ± 16	141 ± 7	6.7 ± 0.8	3.49 ± 0.09
MONO	6.1 ± 1.7	375 ± 38	42 ± 7 (11 ± 1)	251 ± 39 (62 ± 4)	83 ± 9 (27 ± 4)	13 ± 3	263 ± 16	143 ± 8	6.5 ± 0.9	3.45 ± 0.10
POLY	5.8 ± 1.3	370 ± 42	43 ± 9 (10 ± 1)	254 ± 38 (64 ± 4)	75 ± 9 (26 ± 4)	11 ± 2	239 ± 17	142 ± 11	7.5 ± 1.1	3.36 ± 0.12

All values, mean ± SEM. Values in parentheses are percentages.

nificant differences between monounsaturated and saturated fat groups were only found during the first 9 weeks. The values for the saturated fat group in period 2 decreased dramatically at 12 and 14 weeks into the study because the cholesterol was accidentally omitted from the diet fed to this group during this period. When this problem was corrected, values returned to a plateau similar to or slightly higher than that at 9 weeks into the study. On the plateau, the average TPC values for the three groups were different from each other as they were early in the study, although the variance was somewhat greater (for weeks 31, 40, and 48, significance as determined by one-way ANOVA was $P = 0.041$, 0.013, and 0.052, respectively).

Fig. 3 was drawn to make it possible to directly compare the data between periods 1 and 2, given that the dietary fatty acid compositions were different during these periods. For the saturated fat group, TPC values at 8, 22, and 40 weeks were significantly higher ($P < 0.05$, $P = 0.02$, and $P = 0.005$, respectively; paired t test) for period 2 than for period 1. For the monounsaturated fat group during weeks 4 through 13, TPC values were higher ($P < 0.05$) in period 1 than period 2, while TPC values at 18, 22, 31, and 40 weeks were not different between the two periods. For the polyunsaturated fat groups, the values for period 1 after 4 weeks were all significantly higher ($P < 0.001$) than the corresponding values during period 2 except at 40 weeks where the significance level was $P = 0.08$.

The HDL cholesterol values for the two dietary periods are plotted in **Fig. 4.** For both time periods, the HDL cholesterol concentrations are significantly ($P < 0.01$) lower in the polyunsaturated fat group than in the other two groups throughout the study period. The HDL cholesterol concentrations are equivalent for the saturated and monounsaturated fat groups in both diet periods. The trend for HDL cholesterol concentrations to increase after the monounsaturated and saturated fat are begun and then to decrease is real and reproducible (and was seen during the initial challenge in Fig. 1). However, the subsequent decrease appears to have been attenuated by the higher dietary monounsaturated fatty acid content of period 2. The HDL cholesterol concentrations were sig-

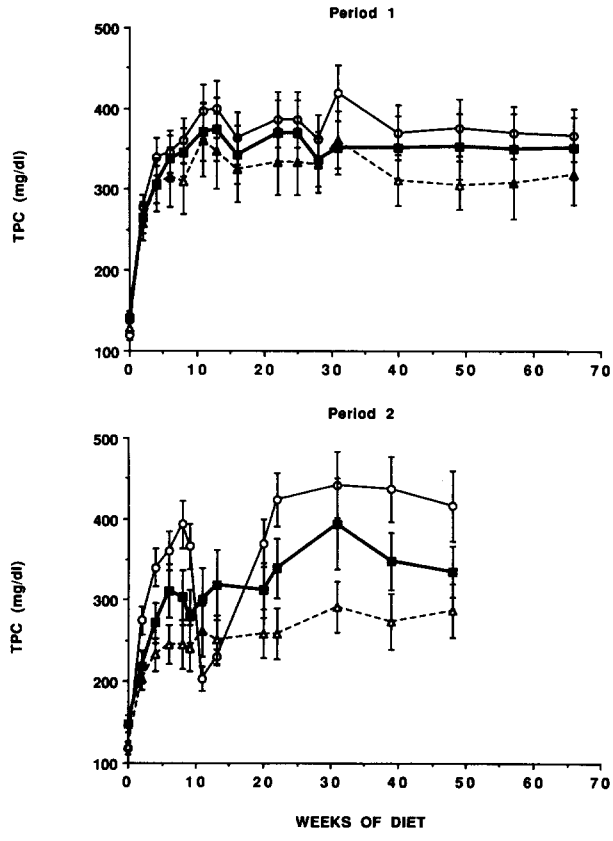

Fig. 2. Time course of TPC response to diets with different fat types during period 1 (upper panel) and period 2 (lower panel). The means ± SEM for the groups fed saturated (open circles), monounsaturated (closed boxes), and polyunsaturated (open triangles) fat are shown. The values at zero were taken before the diet was fed on the first day of the period; the monkeys had been eating monkey chow for the preceeding 6 weeks. Within a dietary fat group, the same monkeys were present during periods 1 and 2.

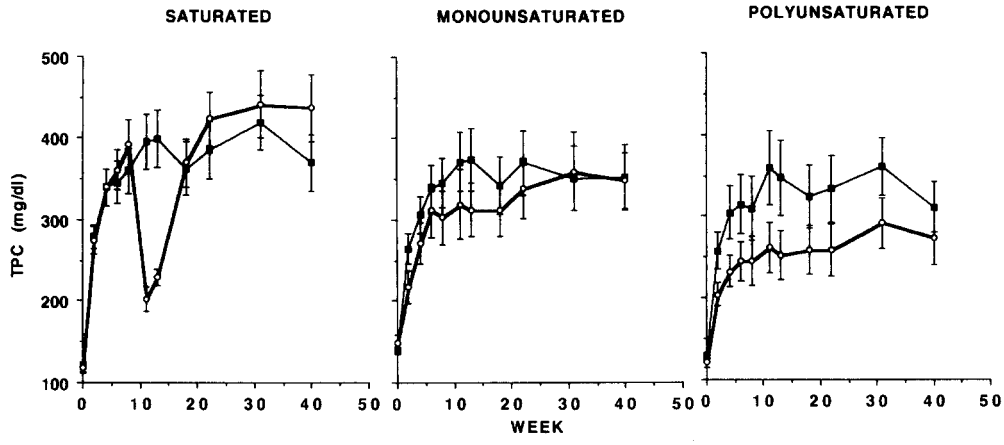

Fig. 3. Time course of TPC response to diets with different degrees of enrichment of saturated, monounsaturated, and polyunsaturated fat during period 1 (closed boxes) and period 2 (open circles). The same animals were fed during each period and the data for the first 40 weeks of each period are shown. All values are means ± SEM.

nificantly higher for both the saturated fat and monounsaturated fat groups compared to those of the polyunsaturated fat group, and this was true during both period 1 and period 2. No differences in HDL cholesterol concentrations were found between period 1 and 2 for the polyunsaturated fat group.

At 9 weeks into each of the experimental periods, a larger blood sample was taken and a more detailed lipoprotein analysis was performed and apolipoprotein concentrations were measured. The data for period 1 are shown in **Table 4.** The only significant differences among the experimental groups were in the HDL cholesterol and apoA-I concentrations, which were significantly lower in the polyunsaturated fat group than in the saturated fat and monounsaturated fat groups. Although trends were apparent for LDL and whole plasma cholesterol to be lower in the polyunsaturated and monounsaturated fat groups, statistically significant differences were not found.

During diet period 2, many differences were observed as shown in **Table 5.** Statistically significant diet effects on cholesterol concentrations in whole plasma, VLDL+ILDL, LDL and HDL cholesterol concentrations were found. For the most part, these were due to lower values in the polyunsaturated fat-fed animals compared to the saturated fat-fed animals. The LDL cholesterol concentration of the monounsaturated fat group also was significantly lower than in the saturated fat group, as was the whole plasma cholesterol concentration. The percentage of whole plasma cholesterol present in the LDL was lower in the monounsaturated fat group than in either the polyunsaturated or saturated fat group. While there was no difference in the HDL cholesterol concentration between the saturated and monounsaturated fat groups, the percentage in HDL was significantly higher in the monounsaturated fat group.

While there were statistically significant differences among diet groups in LDL cholesterol concentration, no significant differences in the apoB concentration in whole plasma were found. Statistically significant differences in the average LDL particle size were found among diet

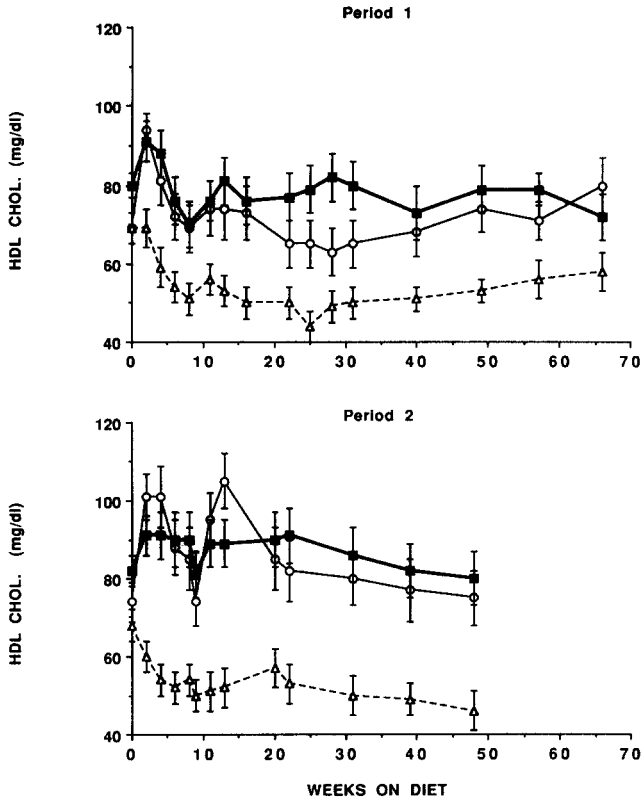

Fig. 4. Time course of plasma HDL cholesterol response to diets with different fat types during period 1 (upper panel) and period 2 (lower panel). The means ± SEM for the groups fed saturated (open circles), monounsaturated (closed boxes), and polyunsaturated fat (open triangles) are shown. The values at zero were taken before the diet was fed on the first day of the period; the monkeys had been eating monkey chow for the preceeding 6 weeks. Within a dietary fat group, the same monkeys were present during periods 1 and 2.

TABLE 4. Dietary effects on plasma lipids, lipoproteins, and apolipoproteins in period 1

Group	Cholesterol: Whole Plasma	Cholesterol: V + ILDL	Cholesterol: LDL	Cholesterol: HDL	TG	ApoA-I	ApoB	ApoE	LDL MW
				mg/dl					*g/μmol*
SAT	330 ± 28	34 ± 3 (16 ± 4)	233 ± 29 (61 ± 6)	65 ± 6 (23 ± 4)	15 ± 3	225 ± 15	118 ± 6	6.8 ± 0.8	3.39 ± 0.08
MONO	309 ± 30	35 ± 5 (11 ± 1)	211 ± 29 (64 ± 4)	64 ± 6 (25 ± 4)	13 ± 2	248 ± 11	119 ± 12	6.1 ± 0.7	3.42 ± 0.09
POLY	299 ± 40	35 ± 6 (11 ± 1)	218 ± 36 (68 ± 4)	48 ± 4[a] (21 ± 3)	12 ± 4	182 ± 9[b]	107 ± 11	6.2 ± 0.8	3.30 ± 0.13

All values, mean ± SEM. Values in parentheses are percentages.
[a]Significantly different from SAT and MONO, $P < 0.02$ and $P < 0.03$, respectively.
[b]Significantly different from SAT and MONO, $P < 0.007$ and $P < 0.0001$, respectively.

groups, with the smallest LDL particles being found in the polyunsaturated fat group and with the largest particles being seen in the saturated fat group. The fact that the LDL particles were larger in the saturated fat group than in the polyunsaturated fat group may be a partial explanation for the larger difference in LDL cholesterol than apoB between these two groups. However, in the monounsaturated fat group, the LDL particles also were larger than in the polyunsaturated fat group and the LDL cholesterol concentrations were equivalent. These data suggested that LDL apoB concentration should be even lower in the monounsaturated fat group than in the polyunsaturated fat group, although such an outcome was not apparent in the whole plasma apoB data. On the other hand, the data for LDL apoB, determined on a separate plasma sample taken at a later time, did show this trend (data not shown).

A significant diet-related difference in apoA-I concentration was also found, but the degree of difference between diet groups was not as marked as for HDL cholesterol. The primary factor in this effect was the difference between the monounsaturated and polyunsaturated fat groups. Interestingly, there also was a significant diet effect on plasma apoE concentration, with the largest difference being between the saturated and monounsaturated fat groups. For the period 2 data, as for the data for period 1, plasma triglyceride concentrations were low among all groups and statistically significant differences were not found.

DISCUSSION

These studies were done in the African green monkey to determine whether monounsaturated fat-enriched diets

TABLE 5. Dietary effects on plasma lipids, lipoproteins, and apolipoproteins in period 2

Group	Cholesterol: Whole Plasma	Cholesterol: V + ILDL	Cholesterol: LDL	Cholesterol: HDL	TG	ApoA-I	ApoB	ApoE	LDL MW
				mg/dl					*g/μmol*
SAT	337 ± 25	43 ± 4 (13 ± 1)	222 ± 24 (62 ± 4)	76 ± 6 (25 ± 1)	23 ± 3	225 ± 20	119 ± 14	7.2 ± 1	3.58 ± 0.03
MONO	256 ± 29	36 ± 5 (14 ± 2)	141 ± 29 (49[a] ± 5)	83 ± 6 (37[b] ± 4)	21 ± 4	244 ± 16	94 ± 13	4.5 ± 0.5	3.50 ± 0.04
POLY	219 ± 24	26 ± 4 (12 ± 1)	145 ± 24 (62 ± 4)	52 ± 4 (26 ± 3)	13 ± 3	182 ± 13	90 ± 12	4.8 ± 0.5	3.36 ± 0.05
One-way ANOVA or ANCOVA[c]									
Diet	0.0002	0.0198	0.0001[c]	0.0001[c]	0.108	0.043	0.25	0.022	0.0008
SAT-POLY	0.0006	0.058	0.0013	0.0007					0.0003
SAT-MONO	0.009		0.0001					0.066	0.079
MONO-POLY				0.0001		0.086			0.03

All values, mean ± SEM. Values in parentheses are percentages.
[a]Significantly different from SAT and POLY.
[b]Significantly different from SAT.
[c]Analysis of covariance using TPC as the covariate.

could be demonstrated to have effects on plasma lipoproteins similar to those seen in humans. Effects on atherosclerosis, per se, will subsequently be documented. We have previously reported that dietary polyunsaturated fat compared to saturated fat in this species lowers plasma LDL and HDL cholesterol concentrations to an extent similar to that seen in humans (16, 20). We also have found that African green monkeys fed polyunsaturated fat-enriched diets had less atherosclerosis than monkeys fed saturated fat-enriched diets (20). In the present study, we compared the monounsaturated fat-enriched diet with polyunsaturated and saturated fat-enriched diets. While the absolute concentrations of HDL cholesterol in this monkey model are somewhat higher than in humans, the relative effects seen in this study were similar to those seen in human subjects by Mattson and Grundy (8) who fed the same dietary fats as those used in period 2 of this study. LDL cholesterol concentrations were lower in monkeys fed a monounsaturated fat compared to a saturated fat diet, and were comparable in the monounsaturated fat and polyunsaturated fat groups. At the same time, HDL cholesterol concentrations were lower in monkeys fed polyunsaturated fat compared to saturated fat, but HDL cholesterol concentrations in monkeys fed monounsaturated fat were the same as those in monkeys fed saturated fat. The result of these changes was that the monounsaturated fat-fed monkeys had the lowest percentage of whole plasma cholesterol in LDL, which could be expected to result in the least amount of atherosclerosis in this dietary group. It should be reiterated that the level of dietary cholesterol used in the monkeys of these studies is equivalent among fat groups and higher than in the typical North American human (0.8 vs ~0.25 mg/kcal, respectively). The higher intake in monkeys was needed to get plasma cholesterol concentration averages of these monkeys into the 300 mg/dl range, a target range permitting atherosclerosis evaluation in monkeys and representing high risk in humans.

These observations define the African green monkey as a relevant model for the human situation and an appropriate model in which to determine *1*) the effects of dietary monounsaturated fat on atherosclerosis, and *2*) the mechanisms of dietary fatty acid effects on lipoproteins. Since we have seen that polyunsaturated fat decreases atherosclerosis in this species (20), a finding related to the modified LDL composition and concentrations and one that occurs in spite of lower plasma HDL concentrations, it will be important to determine whether animals fed monounsaturated fat have even less atherosclerosis than those fed polyunsaturated fatty acids. On the other hand, average LDL size has been a parameter highly correlated with coronary artery atherosclerosis in these monkeys (20, 28), and the animals fed monounsaturated fat had larger LDL than those fed polyunsaturated fat. Therefore, it is also a possibility that atherosclerosis may be greater in the monounsaturated fat group compared to the polyunsaturated fat group, although the plasma lipoprotein parameters predict that both of these groups will have less atherosclerosis than the saturated fat group.

It was interesting that the differences among dietary fatty acid groups seen in diet period 2 were more marked than those seen during period 1. The differences in fatty acid compositions between the diet periods were statistically significant although not dramatic (Table 2). The saturated fat diet of period 2 had more palmitic acid and less stearic acid, otherwise the differences were small. The serum cholesterol concentrations for the saturated fat group during period 2 were higher than in period 1, suggesting that this difference in fatty acid composition had a real effect. The monounsaturated fat diet had significantly more palmitic acid and less oleic acid in period 1 than in period 2, and the serum cholesterol concentrations were higher during the first 13 weeks of period 1. These differences did not hold up during the later times of the feeding period, however, when cholesterol concentrations were similar. The change in the polyunsaturated fat diet from period 1 to period 2 resulted in the largest difference in dietary fatty acid percentage among the study groups. For the diet of period 2, lower percentages of palmitic acid and oleic acid and a higher percentage of linoleic acid were found for the polyunsaturated fat group and, not surprisingly, the total plasma cholesterol was lower throughout period 2. In each of these dietary comparisons, plasma cholesterol was lower in the period in which the percentage of palmitic acid was less. For the group fed the monounsaturated fat diet, plasma cholesterol was lower when the percentage of dietary oleic acid was higher. For the group fed the polyunsaturated fat diet, plasma cholesterol was lower when the percentage of linoleic acid was higher.

These changes are all in the direction predicted from previous studies with these fatty acids, but we had not anticipated finding such clear effects between the diets of periods 1 and 2, since differences among groups in diet period 1 were not statistically significant although they already represented major differences in fatty acid composition. This type of finding suggests that fatty acid effects have a threshold and, beyond the threshold, the effect is clearly demonstrated. Another possibility is that the effect is a cumulative one, and the differences of period 2 appeared as a 'prior diet' effect from having fed the animals similar diets in period 1. We tend to disregard this idea since we continued the animals for over 60 weeks in period 1 without a statistically significant effect being seen. Another aspect of these studies that should be emphasized is that, for the monounsaturated fat group, the difference in TPC between periods 1 and 2 disappeared after the animals had been fed their diets for about 3 months. The reason for this outcome is unknown but it

does appear that monounsaturated fatty acids may have short-term effects that are not fully maintained for longer time periods. This is a potentially important observation when one considers the average length of time of the typical study in human subjects.

The fact that statistically significant diet effects on whole plasma and LDL cholesterol concentrations were not seen until the pure fat diets of period 2 were fed poses an interesting dilemma. Is it likely that the exaggerated fatty acid differences are necessary for the real effect of fatty acid composition to occur, or are the less dramatic differences seen in period 1 (when the dietary fatty acid compositions are not so markedly different) an indication of the fact that dietary fatty acid effects are occurring even in this case? Our study does not allow us to learn the answer to this question. However, studies in human beings in which the fatty acid differences were not as marked as in this study have been published (11, 12) and the effect of monounsaturated fat to lower whole plasma and LDL cholesterol concentrations was still present. On the other hand, earlier studies using monounsaturated fatty acids appeared to show minimal effects on total serum cholesterol concentrations (3, 4). While it is not possible to resolve this dilemma at this time, the bulk of the more recent evidence suggests that there is a real effect of monounsaturated fat to lower LDL cholesterol concentrations without lowering HDL cholesterol concentrations, and based on the variety of studies done, it appears likely that this effect is not limited to the situation where pure fats are used. One must remember that most of the intervention studies completed, however, are short term studies, and our data suggest that longer term effects of dietary monounsaturated fatty acids may not be the same. Likewise, one should note that the studies in monkeys were done with higher dietary cholesterol intake and with somewhat higher plasma cholesterol concentrations than has been the case for the studies in humans.

The pre-selection of experimental groups according to diet responsiveness was done to maximize our chances of detecting significant diet effects on atherosclerosis and on lipoprotein endpoints with animal groups of this size and variability. A precedent for evaluating individual animal variability in the plasma lipid and lipoprotein response to an atherogenic diet has been published (16). It was shown that for whole plasma cholesterol concentrations, the rank order correlation among groups of African green monkeys was greater than $r = 0.9$ when the animals were fed three different diets containing saturated or polyunsaturated fat. Therefore, the group selection process used here is likely to equalize the variability among groups in a meaningful way that is not time- and diet-dependent.

The authors wish to thank Cathy Andresen, Denise Boyles, and Rusty Washburn for excellent technical assistance. The dietary oils used in these studies were generously supplied through the good offices of Dr. Ed Hunter representing the Institute of Shortening and Edible Oils, Inc. This work was supported by NIH grant HL 24736.

Manuscript received 2 April 1990 and in revised form 29 June 1990.

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