Reduction of cholesterol absorption by dietary oleinate and fish oil in African green monkeys

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Abstract To determine whether diets enriched in monounsaturated or n–3 fatty acids cause a reduction in cholesterol absorption relative to those more enriched in saturated fatty acids, we measured cholesterol absorption in 18 African green monkeys fed diets enriched in lard, oleinate (oleic acid-rich safflower oil), or fish oil at two levels of dietary cholesterol (0.05 vs. 0.77 mg/kcal). All animals were initially challenged with the lard, high cholesterol diet to ascertain their responsiveness to dietary cholesterol. Based on the results of this challenge, low versus high responders were equally distributed in assignation to the low (n = 6) and high (n = 12) cholesterol regimens. Within each level of dietary cholesterol animals consumed all three dietary fats in random sequences during three experimental phases each lasting 9–12 months with a monkey chow washout period between each phase, so that each animal served as its own control. During each dietary phase measurements of plasma lipids and cholesterol absorption were performed. The animals fed the higher versus lower level of dietary cholesterol had significantly higher plasma total cholesterol and low density lipoprotein (LDL) cholesterol concentrations and lower percentage cholesterol absorption; high density lipoprotein (HDL) cholesterol levels were not affected by the level of dietary cholesterol. Dietary fish oil resulted in a 20–30% reduction ($P < 0.01$) in total plasma and LDL cholesterol and a 30–40% reduction ($P < 0.01$) in HDL cholesterol concentrations compared to lard and oleinate regardless of the level of dietary cholesterol. At the high level of cholesterol intake, the oleinate and fish oil diets resulted in significantly lower percentage cholesterol absorption compared to the lard fat diet ($35 \pm 2\%$, $34 \pm 3\%$, $41 \pm 4\%$, respectively). At the lower level of dietary cholesterol, percentage cholesterol absorption values were higher than those at the high cholesterol intake (45–52% vs. 34–41%) but were not affected by the type of dietary fat. There was a significant positive correlation between plasma LDL cholesterol concentrations and percentage cholesterol absorption for the oleinate and lard diets at the high level of dietary cholesterol and a significant inverse association between plasma HDL cholesterol and percentage cholesterol absorption. We conclude that the type of dietary fat can influence cholesterol absorption in African green monkeys and that oleinate and fish oil reduce cholesterol absorption relative to lard when a high amount of cholesterol (0.77 mg/kcal) is present in the diet.—**Parks, J. S., and J. R. Crouse.** Reduction of cholesterol absorption by dietary oleinate and fish oil in African green monkeys. *J. Lipid Res.* 1992. **33:** 559–568.

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Interest in the quantitative effect of the type of dietary fat on plasma lipids started almost 40 years ago when Ahrens et al. (1) and Kinsell et al. (2, 3) demonstrated that vegetable oil could reduce plasma lipid concentrations in man. Subsequent studies by Keys, Anderson, and Grande (4) and Hegsted et al. (5) found that dietary saturated fatty acids (particularly lauric, myristic, and palmitic) raised, n–6 polyunsaturated fatty acids lowered, and monounsaturated fatty acids had no effect on total plasma cholesterol concentrations. A more recent study by Mattson and Grundy (6) reported that oleinate-rich diets lowered total plasma and LDL cholesterol but not HDL relative to lard. Since LDL concentrations are positively correlated (7) and HDL concentrations are inversely associated (8) with the incidence of coronary heart disease, a knowledge of how different types of dietary fats influence plasma lipoprotein concentrations is important to the understanding of coronary heart disease and may provide insights into its prevention.

The mechanisms by which the type of dietary fat alters plasma lipoprotein concentrations are poorly understood. Although intestinal absorption of cholesterol is thought to be at least one regulator of plasma lipoprotein concentrations, there is little evidence that the type of dietary fat influences intestinal cholesterol absorption (9–11). Our recent studies in African green monkeys suggested that cholesterol absorption might be affected by diets enriched in fish oil relative to lard

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein.

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since plasma LDL and HDL and tissue (i.e., liver, abdominal aorta) cholesterol concentrations were lower and secretion of biliary cholesterol during recirculating liver perfusion was unaffected (12–16). However, there is a paucity of data regarding the effect on cholesterol absorption of certain types of dietary fat such as oleinate and fish oil, which are currently being considered for therapeutic benefit with regard to coronary heart disease.

The purpose of this study was to determine whether dietary oleinate and fish oil reduced cholesterol absorption compared to lard in African green monkeys. This animal model was chosen because our previous studies have shown that it is more similar to humans than other species of nonhuman primates with regard to plasma lipoprotein concentrations and distribution (17, 18), response to polyunsaturated dietary fat (19, 20), cholesterol absorption (21), and the types of atherosclerotic lesions that develop with consumption of an atherogenic diet typical of that consumed by most North Americans (22). We found that both oleinate and fish oil reduce cholesterol absorption when fed with a high level of dietary cholesterol.

METHODS

Animals

Eighteen adult African green monkeys (*Cercopithecus aethiops*) were used for this study; 14 of the monkeys were purchased from the Hahnemann breeding colony (Philadelphia, PA) and 4 were from Charles River Imports (Port Washington, NY). The colony included three subspecies: 6 vervets, 8 grivets, and 4 St. Kits. Thirteen of the monkeys were male and 5 were female. Animals were housed in individual cages throughout the study period. The protocol for this study was approved by the Institutional Animal Care and Use Committee.

Diets

The animals were fed low (0.05 mg cholesterol/kcal) or high (0.76 mg cholesterol/kcal) cholesterol diets containing 40% of calories as fat with 22% of calories as lard, menhaden oil, or oleic acid-rich safflower oil (oleinate). Diet compositions are given in **Table 1.** Each diet contained 11 g of the principle test fat with additional cholesterol added in the form of dried egg yolk in the high cholesterol diets. For the control diets, egg yolk replacement (a low cholesterol substitute for egg yolk) was added to balance the protein, carbohydrate, and fat content of egg yolk as well as the fatty acid composition (13). Crystalline cholesterol and β-sitosterol were added to diets as indicated to balance the sterol content of the diets. On average, the low cholesterol group consumed 37 mg cholesterol/day while the high cholesterol group received 560 mg/day. All other dietary constituents were the same among the diets. The fish oil diet provided ~3.8 g of n–3 fatty acids per day. Antioxidants (Tenox 20A) and vitamin E were added to the saturated and monounsaturated diets to balance the amounts added to the processed fish oil.

Diets were made in 10-kg batches and stored frozen until use. Frozen diet was allowed to thaw in a refrigerator and was fed to animals within 24 h after removal from the freezer. The total amount of food given each day was equivalent to 134 kcal/kg body weight. Animals were trained to consume two meals daily, each of 30 min duration. Uneaten diet was removed from the animal cages after the 30-min meal to minimize the risk of diet oxidation. Aliquots of the diets were analyzed for fatty acid composition after extraction with chloroform–methanol 2:1 (12). Water was provided to the animals ad libitum.

The fatty acid composition of the experimental diets is given in **Table 2.** For these data six to eight batches

TABLE 1. Diet composition (g/100 g)[a]

	Lard	Oleinate	Fish Oil
Lard	11		
Oleinate[b]		11	
Menhaden oil[c]			11
Egg yolk or replacement[d]	15	15	15
Cholesterol	0.0066	0.0176	
β-Sitosterol	0.035		0.035
α-Tocopherol	0.0164	0.0128	
Tenox GT-2	0.0132	0.0128	
Tenox 20A	0.011	0.011	
	Same for all		
Casein		9.0	
Lactalbumin		5.0	
Wheat Flour		35.0	
Sucrose		12.0	
Alphacel		7.0	
Ausman-Hayes Mineral Mix		3.5	
Complete Vitamin Mix		2.5	
D_3 in corn oil		6.25 ml	

[a]All diets had a caloric distribution of 19% protein, 42% fat and 39% carbohydrate. All diets provided 450 kcal/100 g dry weight.

[b]Oleinate is oleic acid-rich safflower oil and was furnished through the Institute of Shortening and Edible Oils (Washington, DC).

[c]Processed menhaden oil (vacuum-stripped) was supplied by the Southeast Fisheries Center (Charleston, SC) through the Nutrition Committee Fish Oil Test Material Program at the NIH. The fish oil contained 1 mg/g oil of the following antioxidants: α-tocopherol, gamma-tocopherol (Tenox GT-2) and Tenox 20A (20% tertiary butylhydroquinone). These antioxidants were added to the saturated and monounsaturated diets to balance the antioxidant content.

[d]High cholesterol diets contained 15 g of dried egg yolk per 100 g diet as a source of cholesterol; low cholesterol diets contained 15 g of egg yolk replacement per 100 g diet, a low cholesterol substitute that resembles egg yolk in caloric distribution and fatty acid composition (13). Crystalline cholesterol and β-sitosterol were added to balance the sterol content of all diets. Cholesterol content of diet ingredients was as follows: lard, 1 mg/g; menhaden oil, 1.6 mg/g; egg yolk, 22 mg/g and egg yolk replacement, 0.4 mg/g.

TABLE 2. Dietary fatty acid percentage composition

Fatty Acid	Lard (n = 6)	Oleinate (n = 8)	Fish Oil (n = 7)
		(%)	
14:0	1.4 ± 0.1[a]	0.6 ± 0.1	5.0 ± 0.2
16:0	25.2 ± 0.2	14.1 ± 0.1	22.2 ± 0.3
16:1	2.5 ± 0.1	1.1 ± 0.1	7.2 ± 0.1
18:0	13.8 ± 0.4	6.5 ± 0.4	7.6 ± 0.6
18:1	41.4 ± 0.5	59.8 ± 0.7	26.0 ± 0.3
18:2	13.2 ± 0.4	16.9 ± 0.3	9.0 ± 0.4
20:4	0.5 ± 0.2	0.4 ± 0.2	0.9 ± 0.2
20:5 n-3	ND[b]	ND	7.8 ± 0.4
22:5 n-3	ND	ND	1.3 ± 0.1
22:6 n-3	ND	ND	4.5 ± 0.3
Other	2.1 ± 0.1	0.5 ± 0.1	8.5 ± 0.5

[a]Mean ± SEM. Equal number of batches of low versus high cholesterol diets were analyzed within each type of dietary fat except for the fish oil diet in which four high cholesterol and three low cholesterol batches were analyzed.
[b]ND, not detectable.

of diet made over a 1.5-y span were analyzed. Relative to the lard diet the oleinate diet contained significantly less 14:0, 16:0, 16:1, 18:0 and other fatty acids and significantly more 18:1 and 18:2. The fish oil diet contained significantly less 16:0, 18:0, 18:1, and 18:2 and a greater percentage of 14:0, 16:1, 20:5 n–3, 22:5 n–3, 22:6 n–3, and other fatty acids compared to the lard diet.

Experimental design

The experimental design of the study is shown in **Fig. 1.** All animals were given a 6-month high cholesterol-lard dietary challenge at the start of the experiment to assess their degree of responsiveness to the diet. This was done because some animals are high responders to dietary cholesterol challenge (i.e., marked increase in total plasma cholesterol) while others only respond slightly to dietary cholesterol (i.e., low responders) and we wished to assure equal distribution of such responders between groups fed low and high cholesterol diets. Toward the end of the challenge period, total plasma and HDL cholesterol concentrations were determined on three separate occasions over a 2-month period for each animal. The animals were rank-ordered based on the mean value of these three lipid determinations and stratified into six groups of 3 animals each. Within each stratum of 3, animals were randomly assigned to the low cholesterol (1 animal) or high cholesterol (2 animals) regimen of one of the six diet sequences illustrated in Fig. 1. Thus, for each diet sequence, 2 of the 3 animals consumed the high cholesterol diet while 1 animal consumed the low cholesterol diet. The randomization of animals within each stratum assured that equal proportions of low versus high responders were dis-

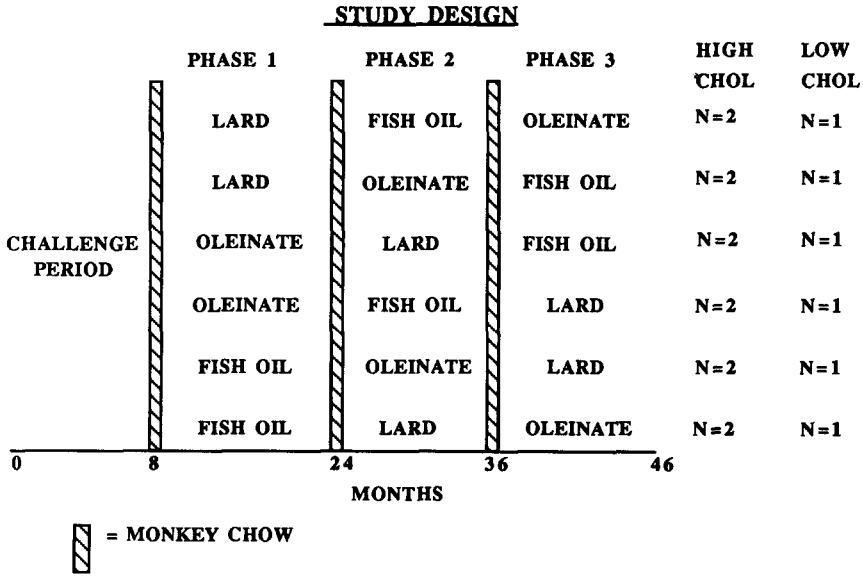

Fig. 1. Diagram of the experimental design of the study. Eighteen adult African green monkeys were subjected to a 6-month challenge to assess responsiveness of plasma lipids to a lard, high cholesterol diet. Six animals were then assigned to low cholesterol dietary regimens so that the mean ± standard deviation for total plasma cholesterol and HDL cholesterol during the challenge phase were not significantly different for the animals assigned to the high versus low cholesterol groups. Within each level of dietary cholesterol animals were fed three types of dietary fat in three experimental phases that lasted from 9 to 12 months so that each animal served as its own control. A 6–8 week monkey chow "wash-out" phase was used between each experimental phase to bring plasma lipids back to baseline. Animals were randomly assigned to one of six possible diet sequences. Periodic blood samples were taken for plasma lipid determinations and cholesterol absorption studies were performed towards the end of each experimental phase.

tributed into low and high cholesterol diet sequences. Six of 18 animals were in the low cholesterol diet sequences because African green monkeys consuming low cholesterol diets have less inter-animal variability in their plasma lipid response than do those consuming high cholesterol diets (19).

A 6-week monkey chow period was used to bring plasma lipid concentrations back to baseline after the challenge period before the Phase 1 diets were started. After the monkey chow "washout" period, the animals were given the next dietary fat in their sequence (i.e., top row of Fig. 1; lard to fish oil to oleinate). Each dietary phase lasted 9–12 months. All animals completed the study. The body weight of the animals averaged 5 kg and did not change more than 4% during the course of the study once the animals had equilibrated to the high fat, cholesterol-containing diets. During each dietary phase periodic blood samples were taken after an overnight fast and ketamine (10 mg/kg) restraint of the animals for determination of total plasma cholesterol, HDL cholesterol, and triglyceride concentrations using Lipid Research Clinic methodology as described previously (14). VLDL + IDL + LDL cholesterol concentrations were calculated as total minus HDL-C and were abbreviated as 'LDL' since the amount of VLDL and IDL cholesterol in the plasma of these animals was < 5% that of the LDL fraction as determined by size exclusion chromatography of plasma lipoproteins and enzymatic cholesterol analysis (J. S. Parks and A. K. Gebre, unpublished data). At the end of each dietary phase a cholesterol absorption study was performed as described below.

Cholesterol absorption studies

Cholesterol absorption was measured by a continuous feeding isotope absorption method previously described (23). [1α, 2α (n) - ^{3}H]cholesterol (45.6 Ci/mmol) and [4 - ^{14}C]β-sitosterol (56 mCi/mmol) were purchased from Amersham (Arlington Heights, Il) and the purity of each was checked by HPLC on a C18 column (Rainin, Woburn, Ma). Thirty μg of unlabeled cholesterol or β-sitosterol was added to the appropriate radiolabel and the mixture was injected onto the column in 70% tetrahydrofuran–30% acetonitrile and eluted with 92.5% acetonitrile–7.5% tetrahydrofuran at a flow rate of 2 ml/min and a column temperature of 35°C. Fractions (0.5 min) were collected and the elution of the radiolabeled materials was compared to the elution of unlabeled, authentic cholesterol or β-sitosterol (absorption at 213 nm). The animals were given 0.5 μCi of [^{3}H]cholesterol and 0.08 μCi[^{14}C]β-sitosterol (dissolved in ethanol and placed on a slice of apple) per day for 6 days just prior to their morning feeding and stools were collected on days 4–7 after initiation of the isotope administration. Stool samples (about 1 g) were saponified with sodium hydroxide in 90% ethanol and extracted with hexane as previously described (24). Hexane extracts were evaporated to dryness then redissolved in less than 0.5 ml of hexane, transferred to ash-free paper cones, and dried on the paper. Samples were combusted in a model 306 Tri-carb sample oxidizer (Packard, Downers Grove, IL) that separates and traps [^{14}C]CO_2 and [^{3}H]H_2O. This equipment was tested for recovery with known standards each time it was used, and recovery in all cases was ≥ 95%. Tritium was measured in Monophase-40 and ^{14}C in Permaflur V in a Packard 1900 CA Tricarb liquid scintillation analyzer. Cholesterol absorption was quantified by comparison of the fecal isotope ratio to that in the diet as previously described (23). The technician who analyzed the fecal samples for cholesterol absorption data was masked as to the sequence of diet fed as well as to the allocation of animals to diet. Daily stool samples were analyzed in duplicate for all 4 days of collection and the eight values for each animal were averaged for each dietary phase. The average coefficient of variation for cholesterol absorption measurements was 12% (n = 54).

Data analysis

In general, data are presented as mean ± standard error of the mean. Two factorial (low vs. high dietary cholesterol), repeated measures (type of dietary fat) analysis of variance was used to test for significant effects of dietary cholesterol level and the type of dietary fat and for interactions between dietary cholesterol and type of fat on plasma lipids and cholesterol absorption. Within a given level of dietary cholesterol, repeated measures analysis of variance was used to test for effects of type of dietary fat on plasma lipids and cholesterol absorption; individual dietary fat differences were determined using a Scheffe F-test post-hoc analysis (25). As animals were randomized into all possible dietary sequences for the three types of dietary fat and all animals completed the three dietary phases, a balanced experimental design was maintained to randomize potential time effects on the measured variables.

RESULTS

Fig. 2 illustrates the individual animal response of total plasma cholesterol and HDL-cholesterol concentrations to the challenge diet. All 18 animals had an increase in total plasma cholesterol when switched from chow to the high cholesterol, lard challenge diet, although the response was variable among animals. Approximately half of the animals responded with

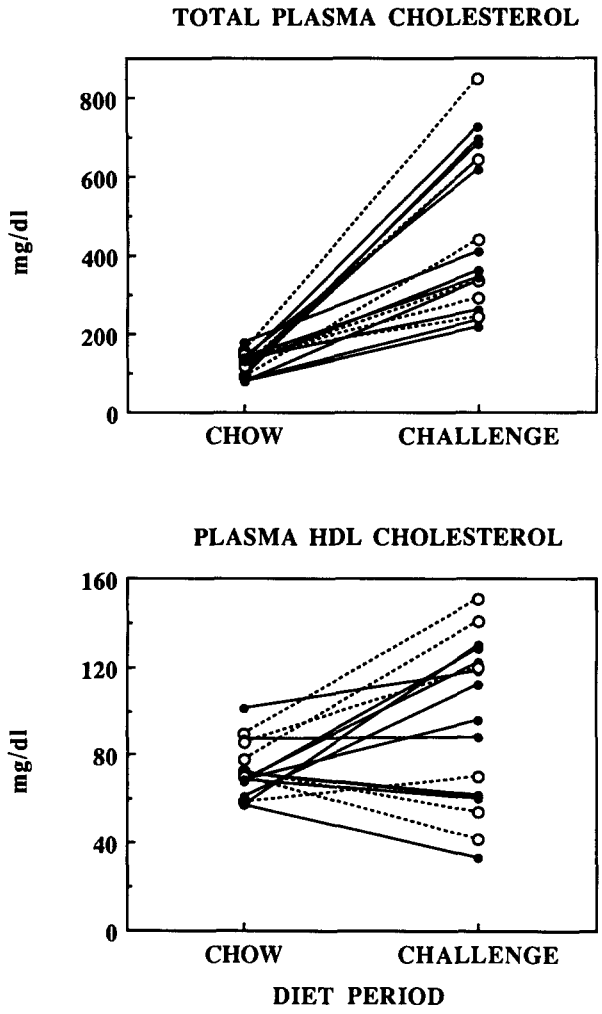

Fig. 2. Total plasma and HDL cholesterol concentrations of individual African green monkeys before (chow) and during the dietary challenge with a lard, high cholesterol diet. After the challenge phase animals (n = 6) represented by the open symbols with dashed lines were assigned to the low cholesterol dietary regimens while those represented by the closed symbols (n = 12) were assigned to the high cholesterol regimens. Challenge values are the mean of three separate determinations for each animal over the last 2 months of the challenge phase.

total plasma cholesterol values ≤ 400 mg/dl and the remainder had concentrations ranging from 400 to 800 mg/dl. HDL-cholesterol responses of the animals to the challenge diet were even more variable than the total plasma cholesterol concentrations; 7 of 18 animals had decreases in HDL-cholesterol when switched from chow to the challenge diet while 10 of the animals had increases in HDL-cholesterol concentrations. HDL-cholesterol concentrations were inversely correlated with total plasma cholesterol concentrations. The mean ± standard deviation for total plasma cholesterol was 467 ± 235 versus 442 ± 200 mg/dl and for HDL-cholesterol was 96 ± 48 versus 87 ± 32 mg/dl for the low (n = 6) versus high (n = 12) cholesterol groups, respectively, based on the challenge values. Thus, the inter-animal variability in plasma lipid response to dietary cholesterol challenge was matched for the low and high cholesterol groups before the experimental phases of the study were initiated (i.e., phases 1–3 in Fig. 1).

The effect of type of dietary fat and level of dietary cholesterol on plasma lipid and lipoprotein concentrations and cholesterol absorption is shown in **Table 3.** The values are the mean ± SEM for the entire study. Increasing the amount of cholesterol in the diet from 0.05 to 0.77 mg/kcal (15 fold) resulted in an average 2.4-fold increase in total plasma cholesterol (130–180 to 311–427 mg/dl) and a 4-fold increase in 'LDL'-cholesterol (65–80 to 261–343 mg/dl). Percentage cholesterol absorption was greater in the low cholesterol diet group (45–52%) compared to the high cholesterol group (32–41%). There was a significant dietary cholesterol × dietary fat interaction for total plasma cholesterol and the percentage cholesterol absorption, and a borderline significant interaction for 'LDL'-cholesterol indicating that the effects of the diets on these variables were dependent on the type of fat as well as the level of dietary cholesterol. Total plasma cholesterol concentrations were significantly lower when fish oil was fed compared to lard or oleinate regardless of the level of dietary cholesterol. However, 'LDL'-cholesterol was significantly lower only when fish oil was fed at the high level of dietary cholesterol. HDL-cholesterol concentrations were significantly lower when animals were fed fish oil compared to lard or oleinate at both levels of dietary cholesterol. The percentage cholesterol absorption was not affected by the type of dietary fat at the low level of dietary cholesterol but was significantly reduced when the animals were fed oleinate or fish oil compared to lard at the high level of dietary cholesterol. Thus, the effect of type of dietary fat on cholesterol absorption was only apparent at the higher level (0.77 mg/kcal) of cholesterol intake.

The plasma lipid and cholesterol absorption response of individual animals on the high cholesterol diets is shown in **Fig. 3.** Relative to the high cholesterol, lard diet, the fish oil diet resulted in lower average total plasma cholesterol concentrations for all 12 animals whereas reductions in plasma cholesterol were variable with the oleinate diet. Similarly, 11 of 12 animals had lower 'LDL'-cholesterol concentrations when consuming high cholesterol, fish oil versus lard diets whereas LDL responses to oleinate were variable. All 12 animals when fed the fish oil diet demonstrated a lower average HDL-cholesterol concentration compared to the lard diet, while the oleinate diet produced a variable response. A lower percentage cholesterol absorption was found for 8 animals while consuming the high cholesterol, oleinate diet and for 10

TABLE 3. Effect of type of dietary fat and level of dietary cholesterol on plasma lipid and lipoprotein concentrations and cholesterol absorption

Dietary Chol. Level	Dietary Fat	TPC	LDL-C	HDL-C	% Chol. Absorption
mg/kcal			*mg/dl*		
0.77	Lard	427[a]	343	84	41
	(n = 12)	±54	±61	±8	±4
	Oleinate	411	327	84	35[c]
	(n = 12)	±48	±55	±8	±2
	Fish oil	311[b]	261[b]	50[b]	34[c]
	(n = 12)	±43	±46	±4	±3
0.05	Lard	178	79	99	45
	(n = 6)	±10	±11	±4	±3
	Oleinate	167	78	90	45
	(n = 6)	±4	±7	±5	±4
	Fish oil	129[b]	65	64[b]	52
	(n = 6)	±13	±12	±8	±4
			ANOVA		
	Chol	0.01	0.01	0.263	0.029
	Type of fat	0.0001	0.001	0.0001	0.215
	Chol × fat interaction	0.03	0.058	0.373	0.024

[a]Mean ± SEM of four-eight observations (after equilibration to diets) for each animal during each dietary phase for total plasma cholesterol (TPC), LDL-C and HDL-C; one observation for each animal during each dietary phase was made for cholesterol absorption. LDL-C was calculated as the difference between TPC and HDL-C.
[b]Significantly different from lard and oleinate diets within level of dietary cholesterol.
[c]Significantly different from lard diet within level of dietary cholesterol.

animals while consuming the high cholesterol, fish oil diets relative to the high cholesterol, lard diet.

The relationship between cholesterol absorption and plasma 'LDL'- and HDL-cholesterol concentrations is shown in **Fig. 4** and **Fig. 5**, respectively. There was a significant positive association between plasma 'LDL'-cholesterol concentrations and percentage cholesterol absorption when animals were fed lard and oleinate at the high level of dietary cholesterol; the association was somewhat weaker ($r = 0.36$) and not statistically significant for the fish oil diet group because one animal had a high level of cholesterol absorption (i.e., 55%). There was an inverse association between plasma HDL-cholesterol and percentage cholesterol absorption when the animals were consuming the high cholesterol, lard and oleinate diets but no such relationship was apparent for the fish oil diet.

DISCUSSION

This study was undertaken to determine whether dietary oleinate and fish oil reduced cholesterol absorption and plasma lipids relative to a lard diet in African green monkeys. Our previous studies demonstrated that the African green monkey, when fed atherogenic diets similar to the ones used in this study, had plasma lipoprotein concentrations and lipoprotein distribution and atherosclerotic lesions that resemble those of humans more closely than any other species of nonhuman primates (17–20). In this study both oleinate and fish oil reduced the percentage of cholesterol absorption compared to lard but only at a high level of dietary cholesterol (0.77 mg/kcal). In addition, there was a significant association between plasma 'LDL'-cholesterol concentrations and percentage cholesterol absorption and a significant inverse association was observed between cholesterol absorption and HDL-cholesterol levels when animals were fed the high cholesterol, lard or oleinate diets. In our previous studies African green monkeys fed a fish oil, high cholesterol diet similar to the one used in this study had reduced plasma, aortic and liver cholesterol concentrations with no difference in biliary cholesterol secretion, during isolated liver perfusion, relative to the lard diet group (12–16). These results indirectly suggested that cholesterol absorption might be influenced by dietary fish oil. The results of the present study suggest that a decrease in cholesterol absorption may have contributed to the lower plasma and tissue cholesterol concentrations in our previous studies.

There is a general belief that the type of dietary fat has little influence on cholesterol absorption in humans (11, 26, 27). However, past studies used less sensitive methods for measuring cholesterol absorption and concentrated on the effect of n–6 polyunsaturated versus saturated dietary fat. Several factors preclude direct extrapolation of our results to human beings. First, the decreased cholesterol absorption

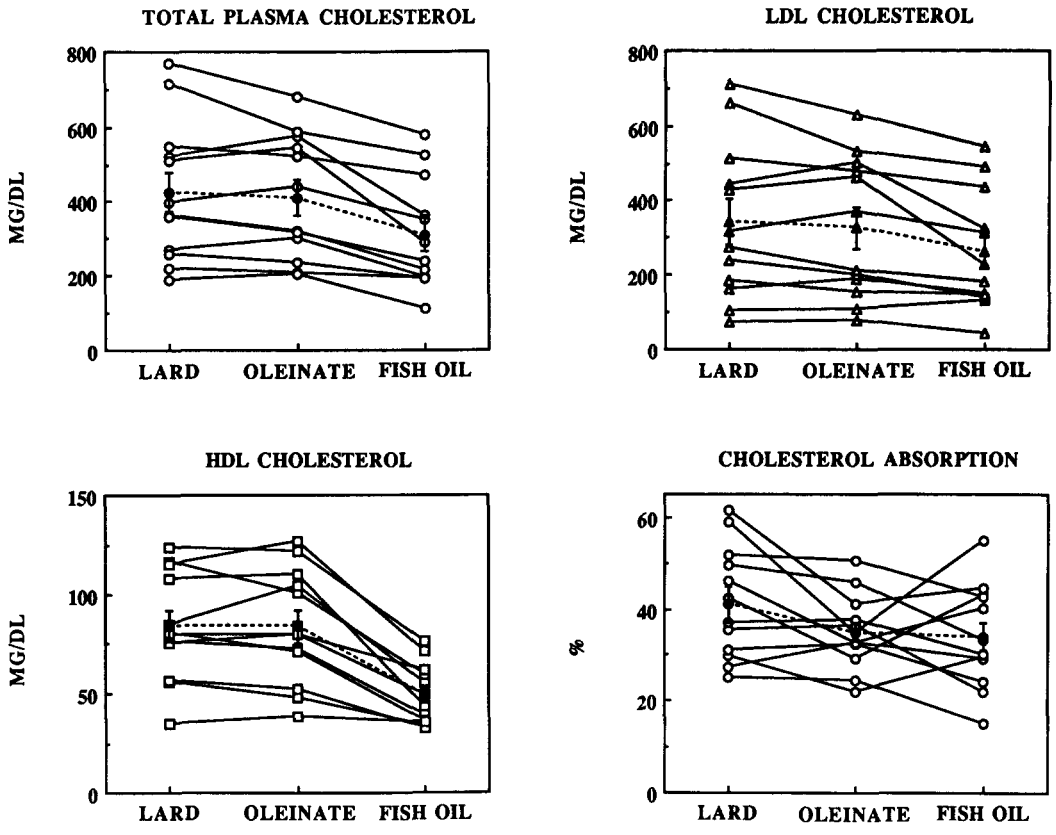

Fig. 3. Response of individual animals consuming the high level of dietary cholesterol to the type of dietary fat. Values with closed symbols represent the mean ± SEM for all 12 animals.

found with dietary oleinate and fish oil was only observed when 0.77 mg chol/kcal was consumed; this amount of dietary cholesterol is considerably higher than that normally consumed by human beings. Second, mixtures of fat rather than pure fats were used in our study so the effects that were observed may be specific for our mixture of dietary fats and not necessarily related to the consumption of monounsaturated or n–3 fatty acids, per se. On the other hand, our results do show that under well-controlled experimental conditions, the long-term consumption of oleinate and fish oil with a high level of dietary cholesterol in our animal model was associated with reduced cholesterol absorption compared to lard. Our data suggest that cholesterol absorption should be examined in human subjects consuming oleinate and fish oil since these fats are being studied as substitutes for saturated fat to help reduce plasma cholesterol concentrations in individuals with hyperlipidemia.

Only a few studies exist on the influence of type of dietary fat on cholesterol absorption and most have suggested that no effect on cholesterol absorption was observed (11, 26, 27). One recent study in rats suggested that fish oil reduces cholesterol absorption, measured as recovery in thoracic duct lymph of radiolabeled cholesterol infused into the duodenum (28). Our results demonstrate a reduction in cholesterol absorption with oleinate and fish oil relative to lard in African green monkeys. The explanation for the reduced cholesterol absorption is not known but is likely to be related to the fatty acid composition of the diet. Relative to the lard diet, the oleinate diet contained about half the amount of 16:0 and 18:0 (Table 2). Palmitic acid is reported to raise plasma cholesterol levels based on the studies of Hegsted et al. (5) and Keys et al. (4). However, stearic acid is thought to be neutral with regard to influencing plasma cholesterol concentrations (4, 29, 30). If palmitic acid raises total plasma cholesterol concentrations partly through increasing cholesterol absorption, then the oleinate diet, which is relatively poor in palmitic acid (Table 2), could have the net effect of reducing cholesterol absorption. The fish oil diet, however, contains nearly as much 16:0 as the lard diet, so the decreased cholesterol absorption in this case may be related to the increased content of n–3 fatty acids in the diet. The mixed micelles in the intestine that contain n–3 fatty acids may not solubilize the dietary cholesterol as efficiently for absorption. If these hypothetical mechanisms were correct then oleinate and fish oil would

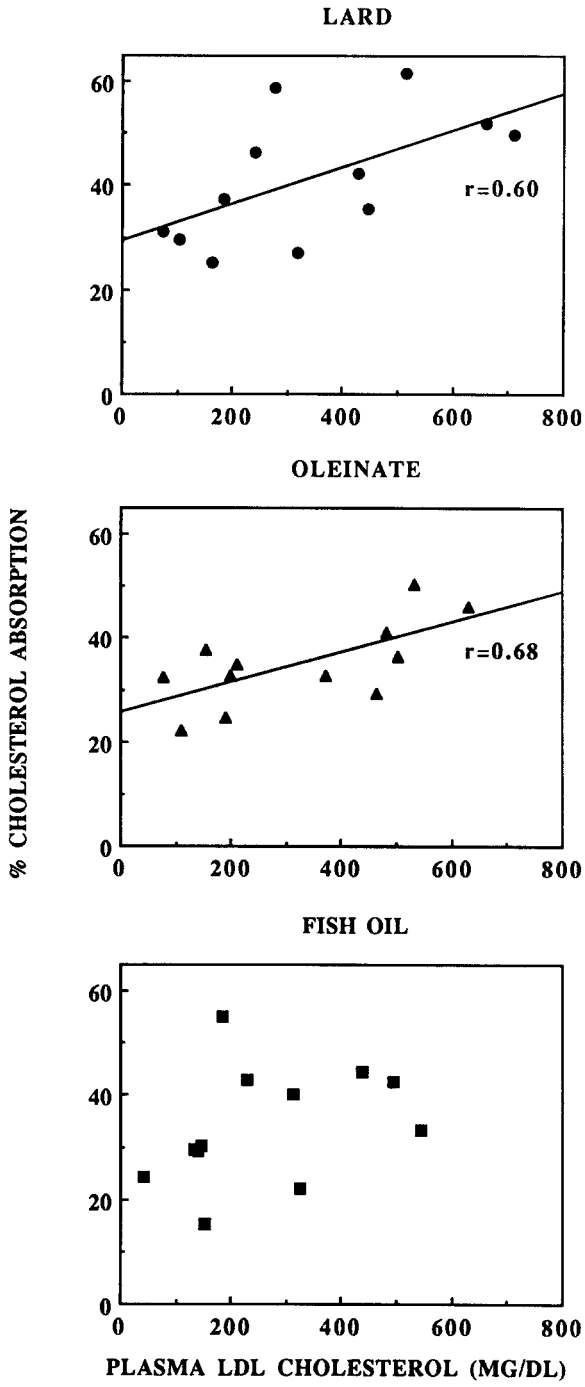

Fig. 4. Relationship between plasma LDL cholesterol concentrations and percentage cholesterol absorption among the high cholesterol diets containing three types of dietary fat. LDL cholesterol was calculated as total plasma – HDL cholesterol. Correlation coefficients were obtained from linear regression analysis. Only statistically significant ($P < 0.05$) correlation coefficients are shown. Each point represents data for one animal including four to eight observations for LDL cholesterol and one observation for cholesterol absorption.

have the same net effect on cholesterol absorption for different reasons. Clearly, more work is necessary to determine the mechanism for the influence of fatty acids on cholesterol absorption.

There was a significant interaction between the level of dietary cholesterol and the type of dietary fat for cholesterol absorption, total plasma cholesterol, and 'LDL'-cholesterol concentrations. This was most clear-

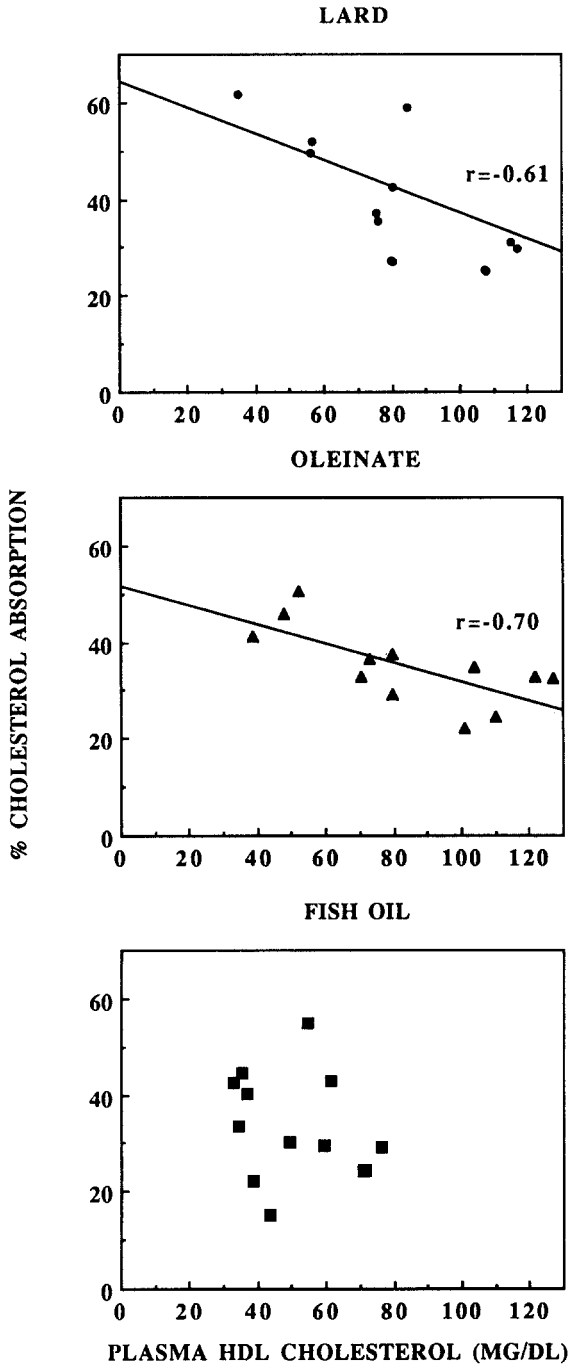

Fig. 5. Relationship between plasma HDL cholesterol concentrations and percentage cholesterol absorption among the high cholesterol diets containing three types of dietary fat. Correlation coefficients were obtained from linear regression analysis. Only statistically significant ($P < 0.05$) correlation coefficients are shown. Each point represents data for one animal including four to eight observations for HDL cholesterol and one observation for cholesterol absorption.

ly demonstrated by the lack of an effect of the type of dietary fat on percentage cholesterol absorption in the animals fed the low amount of dietary cholesterol (Table 3). As the plasma lipid and lipoprotein concentrations of these animals when consuming the low cholesterol diets were similar to those when the animals were consuming monkey chow (compare Table 3 and Fig. 2), it seems likely that most of the absorbed cholesterol was of endogenous origin (i.e., from bile). Perhaps there is a threshold of dietary cholesterol that must be fed to these animals before an influence of the type of dietary fat on cholesterol absorption can be measured. Inasmuch as the percentage cholesterol absorption was significantly higher for the low versus high cholesterol diets, the reduction of cholesterol absorption by oleinate and fish oil may only be apparent when saturating levels of dietary cholesterol are fed to these animals. Additional studies with intermediate levels of dietary cholesterol would be necessary to test this hypothesis.

Studies in human beings have shown that oleinate reduces total plasma cholesterol and LDL-cholesterol concentrations with no effect on HDL-cholesterol levels relative to saturated fat (6). Based on these results it has been suggested that switching dietary fat calories from saturated to monounsaturated sources would be more beneficial with regard to plasma lipoprotein concentrations and coronary heart disease development than switching from saturated to polyunsaturated sources, since high levels of dietary n–6 polyunsaturated fat have been shown to have an HDL-lowering effect (29). Recently, Rudel, Haines, and Sawyer (31) reported that substitution of oleinate for ~60% of the saturated (lard) fat calories in the diet of African green monkeys did not result in a decrease in total plasma cholesterol, a finding similar to that in the present study (Table 3). However, when all of the fat calories were derived from oleinate, a situation similar to the original study of Mattson and Grundy (6), the animals had a significant reduction in total plasma cholesterol compared to a saturated (palm oil) fat group. Thus, our results confirm those of Rudel et al. (31) and suggest that a threshold of oleinate may be necessary to achieve plasma lipid lowering even though in our study cholesterol absorption was significantly reduced when half of the fat calories were derived from oleinate. The fact that cholesterol absorption was affected by oleinate in the animals fed the high level of cholesterol but plasma lipid and lipoprotein concentrations were not suggests that oleinate affects other aspects of cholesterol metabolism that operate to keep plasma cholesterol levels similar to those when lard was fed. This could result from increased hepatic secretion of lipoproteins or reduced receptor clearance of LDL, which might compensate for the reduced absorption of cholesterol during oleinate consumption. Further studies are necessary to test these hypothetical explanations.

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