Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man

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Abstract This study examined the effects of dietary saturated and polyunsaturated fat on the chemical composition and metabolism of low density lipoproteins (LDL) in eight normal male subjects. The influence of these diets on fecal sterol excretion was also measured in four of the subjects. When compared with the saturated fat diet, the polyunsaturated diet lowered both plasma cholesterol (23%, $P < 0.001$) and triglyceride (14%, $P < 0.001$) levels. Sixty-seven percent of the reduction in the former lipid resulted from a fall in LDL cholesterol (23%, $P < 0.001$), although very low density (VLDL) and high density lipoprotein (HDL) cholesterol levels also fell (by 27% and 20% of their respective control value). These changes were accompanied by significant alterations in LDL composition. Specifically, during polyunsaturated fat feeding, the relative percentage cholesterol in the LDL fraction fell while that of phospholipid rose. There was no change in the percentage protein or triglyceride. The fatty acid components of LDL triglyceride, cholesteryl esters, and phospholipid were also affected by dietary fat saturation level. Overall, polyunsaturated fat feeding produced an enrichment in linoleate with reciprocal changes in palmitate, stearate, and oleate which affected triglycerides more than cholesteryl esters and phospholipids. The above changes in LDL composition were associated with alterations in the metabolism of LDL apoprotein (apoLDL). The polyunsaturated diet lowered plasma apoLDL by 13% ($P < 0.05$). This resulted from an increase in the fractional catabolic rate of LDL (whether determined by plasma decay curve analysis ($P < 0.05$) or urine/plasma radioactivity ratios ($P < 0.001$) without significant alteration of its corporeal distribution or synthetic rate. The polyunsaturated fat diet did not cause a consistent change in fecal neutral or acidic steroid excretion. We conclude that the hypocholesterolemic action of polyunsaturated fat diets is effected by multiple mechanisms whose expression may vary from patient to patient.—**Shepherd, J., C. J. Packard, S. M. Grundy, D. Yeshurun, A. M. Gotto, Jr., and O. D. Taunton.** Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J. Lipid Res.* 1980. **21:** 91–99.

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The hypocholesterolemic action of diets rich in polyunsaturated fats has been known for 25 years, but despite intensive studies (see bibliography in (1)), mechanisms for this effect remain obscure. Investigations of the influence of such diets on cholesterol balance in man have revealed several different patterns of response that derive in part from variations in methodology and patient selection. Consequently, these studies have failed to provide a simple explanation based on fecal sterol excretion which accounts for the plasma cholesterol-lowering action of polyunsaturated fats.

Considerably less work has been done on the effects of these fats on lipoprotein metabolism, and studies to date (2, 3) have produced conflicting results. In a recent report (4) we found that 60% of the cholesterol-lowering effect of a polyunsaturated fat diet resulted from a fall in circulating low density lipoprotein (LDL) cholesterol. Since the apoprotein component of LDL (apoLDL) plays an important role in cholesterol transport in man (5), we have pursued this finding in greater detail by examining the influence of the diet on the chemical properties of LDL, on the metabolism of apoLDL, and on cholesterol balance in normal subjects. Our report attempts to define more precisely the mechanism(s) responsible for the polyunsaturated fat-induced reduction of plasma LDL by simultaneous examination of the metabolism of cholesterol and LDL.

Abbreviations: VLDL, very low density lipoproteins, $0.95 < d < 1.006$ kg/l; LDL, low density lipoproteins, $1.019 < d < 1.063$ kg/l; apoLDL, the apoprotein component of low density lipoproteins; HDL, high density lipoproteins, $1.063 < d < 1.21$ kg/l; FCR, fractional catabolic rate.

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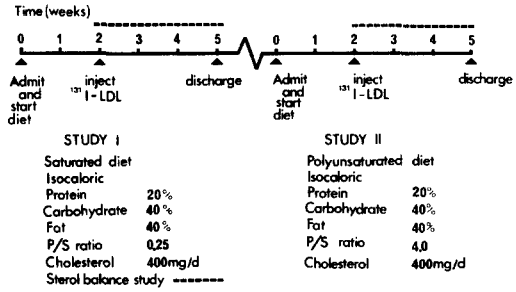

Fig. 1. Plan of metabolic investigation of the effects of saturated versus polyunsaturated fat diets on LDL metabolism and fecal steroid excretion. The interval between the two studies was 8 weeks.

METHODS

Subjects

Eight healthy adult men were studied in the General Clinical Research Center (GCRC) of the Methodist Hospital, Houston, Texas. Their ages ranged from 19 to 23 years, and they were within five percent of their ideal body weight. None had clinical or biochemical evidence of cardiac, hepatic, renal, or endocrine disease; and none were taking medications unrelated to the study. Informed consent was obtained from each patient. The study received the approval of the Human Research Committee of Baylor College of Medicine and the Methodist Hospital.

The experimental design of the study is shown in **Fig. 1.** During Study I, the patients received a diet rich in saturated fats (P/S ratio = 0.25) presented in the form of dairy products (e.g., butter and processed cheese). In Study II the fatty acid saturation level of the diet was altered (to P/S = 4.0) by replacement of the dairy products with safflower oil. Both diets contained 400 mg cholesterol/day and were isocaloric. All food was prepared in the Metabolic Kitchen of the GCRC and was presented on a 2-day cycle. The calorie distribution was 20% as protein (chicken, fish, and specially prepared lean beef), 40% as carbohydrate (cereals, potato, and sugar) and 40% as fat (see above).

LDL turnover and cholesterol balance studies were performed during the last 3 weeks of each diet period, as indicated in Fig. 1. By this time, and in accord with the observations of Spritz and Mishkel (2), plasma cholesterol levels had stabilized and appropriate changes were evident in the fatty acid composition of the plasma lipids.

Each subject received 900 mg of potassium iodide in divided doses for 3 days prior to and throughout the LDL turnover studies to prevent thyroidal sequestration of radioiodide. Chromic oxide (4 × 100 mg daily in divided doses) was also administered during the cholesterol balance study to permit correction for variation in fecal flow. No other medications were given.

Isolation and labeling of LDL

Autologous LDL (d = 1.019 – 1.063 kg/l) was prepared by sequential ultracentrifugation of plasma as described by Langer, Strober, and Levy (6), and labeled with ^{131}I by a modification (6) of the iodine monochloride procedure of McFarlane (7). The ^{131}I-labeled lipoprotein was indistinguishable from unlabeled LDL by previously published criteria (6, 8), and the distribution of radioactivity within the particle was the same on both diets (less than 5% of the lipoprotein radioactivity was extractable with organic solvents and more than 95% of the remaining radioactivity was found in the apoB component of LDL protein and therefore did not penetrate the matrix of a 7.5% polyacrylamide gel on electrophoresis (9).

Turnover of LDL

After two weeks of equilibration of each diet (Fig. 1), the volunteers received an intravenous injection of freshly prepared autologous radiolabeled LDL whose plasma clearance was followed as detailed elsewhere (10). Essentially, the clearance rate of radioactivity from the plasma was measured at daily intervals over a 14-day period and the fractional catabolic rate (FCR) of the ^{131}I-apoLDL tracer obtained by the mathematical procedure of Matthews (11) using the previously validated two-compartment model (6, 10). An independent assessment of the same parameter was made from urine/plasma radioactivity ratios as described by Berson and Yalow (12). Absolute catabolic rates of apoLDL were then calculated using plasma pool sizes for the protein measured by the method of Langer et al. (6). The plasma volume of each subject was taken to be 4% of his body weight.

Lipid analyses

Plasma cholesterol, triglyceride, and lipoprotein cholesterol concentrations were measured at intervals throughout the study by the procedure outlined in the Lipid Research Clinics Manual of Laboratory Operations (DHEW Publication NIH 75-628).

At the end of each diet period and following completion of the turnover studies, LDL (d = 1.019 – 1.063 kg/l) was isolated from 100 ml of fasting plasma and purified by recentrifugation at 1.063 kg/l. This material was free of other lipoproteins and contained less than 1% albumin as judged by immunodiffusion (13).

The isolated LDL was analyzed for free and esterified cholesterol (Boehringer cholesterol kit 15732, Boehringer Mannheim Biochemicals, Indianapolis,

IN), triglyceride (Lipid Research Clinics Manual of Laboratory Operations), phospholipid (14) (as inorganic phosphate, using a correction factor of 25 to express the results as dipalmitoyl phosphatidylcholine) and protein by the Folin phenol method (15). In addition, measurement was made (16) of the fatty acid composition of each lipid class (cholesteryl esters, triglyceride, and phospholipid). The lipids were extracted with 10 volumes chloroform–methanol 2:1 (v/v), and the different lipid classes were separated by thin-layer chromatography on silica gel using an isooctane–ether–acetic acid mixture 75:25:2 (by volume) as solvent. After methylation with a BF_3/methanol mixture, the fatty acid composition of each lipid class was determined by gas–liquid chromatography.

Cholesterol balance studies

Cholesterol balance studies were carried out on four patients as previously described (1). During the last 3 weeks of both periods, all stools were collected and combined into 4-day pools. Fecal neutral and acidic steroids were measured by combined thin-layer and gas–liquid chromatography (17, 18). As noted above, chromic oxide was administered orally as an internal marker to correct for variations in fecal flow (19). No corrections were made for possible degradation of neutral steroids during intestinal transit, (as has been shown to be necessary for patients on formula diets (20), because several investigators (21–26) have presented evidence that losses of neutral steroids are minimal or absent in patients fed solid food diets. It should be noted that the neutral steroid fraction contains both endogenous neutral steroids and unabsorbed dietary cholesterol. The latter usually constitutes about half of the dietary intake (27), and in this study would be approximately 200 mg/day during both diet periods.

RESULTS

As has been shown in earlier studies (4, 28–31), polyunsaturated fat feeding lowered plasma cholesterol and triglyceride (**Table 1**) in this group of normolipemic volunteers. All subjects showed a significant decrease in total plasma cholesterol, ranging from 14–29% (mean 23%). Most (67%) of this reduction derived from a fall in LDL-cholesterol, although VLDL- and HDL-cholesterol also diminished significantly (to 73% and 80%, respectively, of their control values, $P < 0.01$). The plasma triglyceride level of the group fell, on average, by 14% ($P < 0.01$) during polyunsaturated fat ingestion.

The large difference in the saturation level of the

TABLE 1. Effects of dietary fat saturation levels on plasma lipids and lipoproteins

Subject	Age (yr)	Body Weight (n = 36)		Plasma Cholesterol (n = 9)		Plasma Triglyceride (n = 9)		VLDL Cholesterol (n = 6)		LDL Cholesterol (n = 6)		HDL Cholesterol (n = 6)	
		S[a]	P	S	P	S	P	S	P	S	P	S	P
		kg		*mg/dl*		*mg/dl*		*mg/dl*		*mg/dl*		*mg/dl*	
1	22	63.2 ± 0.4	63.0 ± 0.5	171 ± 13	122 ± 9	125 ± 14	109 ± 15	20 ± 5	15 ± 4	109 ± 15	80 ± 6	43 ± 15	27 ± 3
2	21	60.4 ± 0.5	61.7 ± 0.5	185 ± 7	160 ± 12	71 ± 11	69 ± 8	14 ± 5	13 ± 2	135 ± 9	114 ± 13	40 ± 10	36 ± 5
3	19	63.8 ± 0.1	64.5 ± 0.4	205 ± 11	152 ± 11	72 ± 13	63 ± 9	11 ± 3	9 ± 3	148 ± 20	111 ± 9	46 ± 7	31 ± 6
4	21	66.6 ± 0.7	65.4 ± 0.2	211 ± 11	170 ± 6	102 ± 8	88 ± 11	18 ± 4	12 ± 2	147 ± 16	124 ± 4	45 ± 14	32 ± 2
5	23	79.5 ± 0.5	79.1 ± 0.4	161 ± 11	124 ± 11	63 ± 13	50 ± 9	10 ± 2	8 ± 2	104 ± 10	79 ± 6	44 ± 4	41 ± 6
6	22	69.2 ± 0.2	69.8 ± 0.2	239 ± 19	189 ± 8	82 ± 14	70 ± 7	15 ± 4	10 ± 4	174 ± 21	147 ± 4	51 ± 14	34 ± 3
7	21	60.1 ± 0.7	57.8 ± 0.5	173 ± 12	127 ± 7	73 ± 13	61 ± 6	14 ± 2	10 ± 2	110 ± 10	77 ± 9	48 ± 13	41 ± 4
8	22	64.0 ± 0.7	64.3 ± 0.5	222 ± 16	165 ± 17	73 ± 11	56 ± 6	14 ± 4	9 ± 2	143 ± 20	101 ± 10	54 ± 13	56 ± 8
Mean ± 1 SD				196 ± 27	151 ± 25	83 ± 21	71 ± 19	15 ± 3	11 ± 2	134 ± 24	104 ± 25	46 ± 5	37 ± 9
P^b		NS		<0.001		<0.01		<0.01		<0.001		<0.01	

[a] S, saturated diet; P, polyunsaturated diet.
[b] Statistics obtained by paired *t* test.

TABLE 2. Fatt acid analysis of LDL lipids during saturated and polyunsaturated fat feeding

Fatty Acid	Mean Percentage (±1 SD) in Cholesterol Esters			Mean Percentage (±1 SD) in Triglycerides			Mean Percentage (±1 SD) in Phospholipids		
	S[a]	P	Change (%)	S	P	Change (%)	S	P	Change (%)
14:0	2.0 ± 0.7	3.7 ± 2.0	NS[b]	4.8 ± 1.7	6.1 ± 3.2	NS	4.9 ± 2.3	2.6 ± 2.1	47 ($P < 0.01$)
16:0	11.6 ± 1.5	8.8 ± 1.2	24 ($P < 0.02$)	26.5 ± 1.0	15.2 ± 1.6	43 ($P < 0.001$)	26.6 ± 2.0	25.7 ± 1.7	3 ($P < 0.05$)
16:1	2.6 ± 0.6	1.3 ± 0.3	50 ($P < 0.01$)	4.5 ± 0.7	3.1 ± 0.8	31 ($P < 0.01$)	0.7 ± 0.6	—	NS
18:0	1.3 ± 0.1	1.2 ± 0.2	NS	4.6 ± 0.7	3.7 ± 0.8	19 ($P < 0.05$)	13.3 ± 2.4	13.5 ± 2.1	NS
18:1	16.6 ± 1.2	9.1 ± 1.0	45 ($P < 0.001$)	38.6 ± 3.0	26.7 ± 3.1	31 ($P < 0.001$)	13.5 ± 2.1	11.1 ± 1.4	NS
18:2	57.3 ± 2.2	68.7 ± 3.1	20 ($P < 0.001$)	17.8 ± 1.9	41.2 ± 5.7	131 ($P < 0.001$)	27.6 ± 3.4	33.5 ± 3.5	NS
>18:2–<20:4	1.2 ± 1.4	1.9 ± 1.8	NS	1.8 ± 1.3	2.6 ± 1.4	NS	2.9 ± 0.9	3.4 ± 1.3	NS
>20:4	7.2 ± 1.4	5.7 ± 2.7	NS	1.3 ± 1.2	1.4 ± 1.0	NS	10.6 ± 2.3	10.2 ± 3.3	4 ($P < 0.05$)

[a] S, saturated diet; P, polyunsaturated diet.
[b] Statistics obtained by paired *t* test.

two diets was reflected in the fatty acid composition of LDL lipids (triglycerides, cholesteryl esters, and phospholipids) (**Table 2**). As we expected, the greatest alterations were found in the palmitate (16:0), oleate (18:1), and linoleate (18:2) content of the three lipid classes; the percentages of the first two fatty acids were significantly decreased as a result of the polyunsaturated fat diet while linoleate was consistently increased in each lipid class during feeding of this fat. As we had previously observed for HDL (4), the fatty acid composition of LDL-triglycerides was most affected by polyunsaturated-fat ingestion while phospholipids showed the least change.

The relative proportions of the different constituents of LDL were also altered following exchange of polyunsaturated fats for saturated fats (**Table 3**). Polyunsaturated fat feeding caused a consistent (10%) reduction in the percentage of cholesterol in the particle, while the relative percentage phospholipid increased by 14%; both changes were statistically significant ($P < 0.01$). The relative proportions of protein and triglyceride were unaltered. Consequently, the polyunsaturated diet produced a significant decrease in the cholesterol:protein ratio in LDL.

The kinetic parameters of apoLDL metabolism are presented in **Table 4.** All subjects except one (No. 6) showed a fall in the plasma concentration and pool size of apoLDL. The mean reduction in apoLDL concentration was 12.5% which was significant at the 5% level. This fall could not be explained by intercompartmental redistribution of the lipoprotein since the percentage of apoLDL in the intravascular pool was unchanged by the diets.

Synthesis of apoLDL in five of the eight subjects was decreased by polyunsaturated fat feeding, but the reduction for the group as a whole was not statistically significant. On the other hand, polyunsaturated fats appeared to increase the fractional clearance rate (FCR) of apoLDL. This value, as determined by the plasma decay curve of ^{131}I-apoLDL, rose in six of the eight subjects. This increase was statistically significant by paired *t* test ($P < 0.05$). An independent assessment of this value was also obtained from the urine/plasma radioactivity ratios. All eight subjects showed an increased FCR by this method during polyunsaturated fat administration. The increment for the group was highly significant ($P < 0.01$). Additionally, since approximately 14 determinations of the urine/plasma ratio were made for each subject during both phases of the study, the data may be analyzed by pairing the results for each patient in the order of days after injection of the isotope. When this large number of pairs (n = 106) were compared by *t*-test, the dif-

ference between the fractional catabolic rates for the periods of saturated and polyunsaturated fat feeding were highly significant ($P < 0.0001$). Thus, the data demonstrate the consistent finding of an increase in apoLDL FCR during polyunsaturated fat feeding, independent of the method of analysis.

Cholesterol balance data for four subjects are shown in **Table 5.** All measurements were made during a period when LDL-cholesterol concentrations were not changing (steady-state). One subject (No. 2) had a significant increase in fecal neutral steroids on polyunsaturated fats. Otherwise, despite relatively small standard deviations in outputs (i.e., relatively constant daily outputs) none of the other subjects demonstrated increases in neutral or acidic steroids. Thus, during the two study periods, the polyunsaturated fat diet did not effect any increase in fecal steroid excretion.

DISCUSSION

Polyunsaturated fat diets lower the plasma cholesterol concentration, but their mechanisms of action remain controversial, as exemplified by their apparently conflicting effects on fecal steroid excretion (see reference 1). Since we and others (4, 32) have shown that these diets reduce the cholesterol level in all three major lipoprotein fractions (VLDL, LDL and HDL) it is arguable that they may have multiple actions. In the present study we have focused our attention on the influence of polyunsaturated fats on LDL metabolism as measured directly by apoLDL turnover, or indirectly by fecal steroid balance.

Several mechanisms may be proposed to account for the LDL-cholesterol-lowering effect of polyunsaturated fat diets. First, the fractional clearance rate of LDL from the plasma may be enhanced. This suggestion is strongly supported by the results of the present study, which confirms an earlier report of Langer, Levy, and Fredrickson (33). Polyunsaturated fat feeding increased significantly the fractional clearance rate of ^{131}I-apoLDL whether measured by Matthews's (11) analysis of the plasma decay curves or by the independent method of Berson and Yalow (12). The data obtained from the latter method was particularly convincing since it was possible to compare a large number of daily measurements of the FCR for each subject on both diets.

Second, the LDL-cholesterol level in the plasma may fall as a result of decreased synthesis. This mechanism has been suggested in a preliminary report by Turner, Monell, and Brown (3) who studied the influence of polyunsaturated fat diets on apoLDL metabolism in hyperlipidemic subjects using a kinetic technique similar to the one employed here. In our

TABLE 3. Effect of dietary fat saturation level on LDL composition

Subject	Triglyceride		Cholesterol[b]		Phospholipid		Protein		Cholesterol:Protein Ratio	
	S[a]	P	S	P	S	P	S	P	S	P
	%		%		%		%			
1	8.0	11.8	48.6	42.3	24.9	27.3	18.6	18.6	2.61	2.27
2	6.0	6.0	50.3	46.2	24.5	26.8	19.2	21.0	2.78	2.20
3	5.9	7.6	50.4	46.7	25.3	27.6	18.4	18.1	2.74	2.58
4	7.4	9.3	50.7	45.5	23.8	27.6	18.1	17.6	2.80	2.59
5	5.6	7.2	51.0	45.3	25.3	27.1	18.1	20.4	2.82	2.66
6	5.2	5.5	47.8	43.9	26.5	31.8	20.5	18.9	2.33	2.32
7	10.0	6.0	50.8	43.3	20.1	29.6	19.1	21.1	2.65	2.05
8	6.1	5.1	48.0	44.6	28.1	29.1	17.8	21.2	2.70	2.10
Mean ± 1 SD	6.8 ± 1.6	7.3 ± 2.3	49.7 ± 1.3	44.7 ± 1.5	24.8 ± 2.3	28.4 ± 1.7	18.7 ± 0.9	19.6 ± 1.5	2.68 ± 0.14	2.35 ± 0.24
P[c]	NS		<0.01		<0.01		NS		<0.02	

[a] S, saturated diet; P, polyunsaturated diet.
[b] The sum of free and esterified (linoleate) cholesterol.
[c] Statistics obtained by paired t test.
Each assay was performed in duplicate.

TABLE 4. Effects of saturated and polyunsaturated fat diets on kinetic parameters of ApoLDL metabolism

Subject	Plasma apoLDL Concentration		Plasma apoLDL Pool Size[b]		% apoLDL Intravascular		apoLDL Fractional Catabolic Rate				apoLDL Synthetic Rate[e]	
							(a) Calculated[c]		(b) From U/P Ratio[d] (n = 14)			
	S[a]	P	S	P	S	P	S	P	S	P	S	P
	mg/dl		*mg*								*mg/kg/day*	
1	70	59	1990	1674	73	77	0.382	0.408	0.33 ± 0.07	0.39 ± 0.05	12.03	10.84
2	79	74	2146	2055	72	65	0.298	0.346	0.32 ± 0.03	0.36 ± 0.06	10.59	11.52
3	90	72	2583	2088	69	69	0.335	0.333	0.30 ± 0.06	0.32 ± 0.07	13.56	10.78
4	88	81	2637	2382	71	77	0.343	0.316	0.32 ± 0.04	0.33 ± 0.04	13.58	11.51
5	63	52	2255	1851	65	58	0.342	0.397	0.31 ± 0.05	0.35 ± 0.07	9.70	9.29
6	97	106	3022	3330	78	77	0.281	0.296	0.26 ± 0.02	0.31 ± 0.03	12.27	14.12
7	64	54	1732	1405	79	58	0.284	0.345	0.27 ± 0.06	0.33 ± 0.06	8.18	8.39
8	85	63	2446	1823	68	61	0.307	0.366	0.26 ± 0.05	0.31 ± 0.06	11.73	10.38
Mean ± 1 SD	80 ± 13	70 ± 18	2351 ± 407	2076 ± 585	72 ± 5	68 ± 8	0.322 ± 0.035	0.351 ± 0.038	0.30 ± 0.03	0.34 ± 0.03	11.46 ± 1.87	10.85 ± 1.71
P^f	<0.05		<0.05		NS		<0.05		<0.01		NS	

[a] S, saturated diet; P, polyunsaturated diet.
[b] The product of plasma apoLDL concentration and plasma volume (assumed to be 4% of body weight).
[c] Using Matthew's procedure (11).
[d] Ratio of 24 hour urinary radioactivity to mean plasma radioactivity in the same period.
[e] The product of calculated fractional catabolic rate and apoLDL pool size, under the steady-state conditions of the study.
[f] Paired *t* test.

study, five out of eight subjects had lower synthetic rates for apoLDL during polyunsaturated fat feeding, but the mean decrease for the whole group was only 4.5% and was not statistically significant. However, the data do not rule out the possibility that reduced LDL synthesis contributed to the fall in LDL-cholesterol.

Theoretically, changes in either the surface coat or the core of LDL could affect its tissue uptake. Thompson et al. (34) infused a lipid emulsion (Intralipid) or egg lecithin to perturb the saturation level of the phospholipid coat of circulating lipoproteins. It was shown that intravenous infusion of either of the above preparations resulted in an increase in LDL phospholipid saturation and a decrease in the FCR of this lipoprotein as measured by daily ratios of urine/plasma radioactivity. Thus, a change in the composition of phospholipids in the surface coat of LDL may influence the catabolic rate of this lipoprotein. A second study from this laboratory, which probably relates more to changes in the lipid core of LDL particles, was performed by Morrisett et al. (16); they examined the influence of alteration in the dietary fat saturation level on several chemical and physical parameters of the plasma lipoproteins. Two diets of identical composition to those employed in this study were administered. Following a 14-day equilibration period, significant modification of the fatty acid saturation level in LDL triglycerides, cholesteryl esters, and phospholipids was observed. In accord with the present study (Table 2), the magnitude of these changes was greater in the triglyceride fraction than in cholesteryl esters or phospholipid; and in general, polyunsaturated fat feeding increased the linoleate (18:2) content of these lipids and reduced that of palmitate (16:0) and oleate (18:1). The microscopic fluidity of LDL on the polyunsaturated diet was significantly increased, a finding which these workers attributed to the change in the saturation level of LDL lipids. The work of Deckelbaum, Shipley, and Small (35) would implicate an additional potential contributory factor to the change in LDL fluidity, namely, the cholesterol:triglyceride ratio in the lipoprotein. These workers have shown that the fluidity of LDL is strongly dependent on the ratio of these lipids in the core of the particle and can affect the surface properties and hence possibly the metabolism of the lipoprotein (36). A decrease in the ratio, as found during polyunsaturated fat feeding (Table 3), would contribute to an increase in LDL fluidity and, by extrapolation, might promote its catabolism. Although it is difficult to prove this relationship, the results of the present study are in accord with the hypothesis.

A third mechanism by which LDL-cholesterol could be reduced is by modification of the composition of LDL. Such a change was first proposed to explain the action of polyunsaturated fats by Spritz and Mishkel (2). They suggested that these fats altered the configuration of the lipids (cholesteryl esters and phospholipids) within LDL so that the capacity for cholesterol transport is reduced. Their proposal was based on the observation of an increase in cholesterol: protein ratio in LDL during administration of a highly saturated-fat diet, the effect being reversed by feeding polyunsaturates. The present work supports their hypothesis. In all eight of our subjects the cholesterol:protein ratio in LDL was decreased on polyunsaturated fats. This change was accompanied by an increase in phospholipid in the LDL particle. Therefore, polyunsaturated fats change the relative proportions of lipids in the LDL fraction favoring a reduction in its cholesterol content.

A fourth way in which the cholesterol content of LDL might be reduced is by decreased availability for secretion in lipoproteins because of increased excretion from the body, either as cholesterol itself or as bile acids. It has been previously shown by Grundy (1) that patients with hypertriglyceridemia (Types IV and V) have enhanced excretion of both cholesterol and bile acids when polyunsaturated fats are exchanged for saturated fats. Other workers have made similar claims for normal subjects (37). However, Grundy and Ahrens (38) have found that patients with familial hypercholesterolemia, who have normal triglycerides, do not show an increase in fecal steroid excretion on polyunsaturated fats. Our normolipidemic subjects resembled those with hypercholesterolemia in their response. One subject showed a small, significant increase in neutral steroid excretion during polyunsaturated fat feeding but none of the others showed any change in the fecal clearance of either neutral or acidic steroids. Thus, it seems highly unlikely that the low content of cholesterol in LDL caused by polyunsaturated fat feeding can be explained by depletion of hepatic or whole body cholesterol.

In summary, this study indicates that polyunsaturated fats lower LDL-cholesterol concentrations in normal subjects by more than one mechanism. The major contributions to the effect derived from a reduction in the cholesterol content of LDL and an increase in its fractional clearance rate. In a few subjects, a decreased apoLDL synthesis could also have played a role. Furthermore, polyunsaturated fats had other actions in that they lowered both triglycerides and VLDL-cholesterol, and decreased HDL-cholesterol; indeed, on average, reductions in

TABLE 5. Effects of dietary fat saturation on fecal steroid excretion

Subject	Dietary Cholesterol		Fecal Neutral Steroids		Fecal Acidic Steroids		Fecal Total Steroids		Cholesterol Balance	
	S[b]	P	S	P	S	P	S	P	S	P
	mg/day		*mg/day ± 1 SD*		*mg/day ± 1 SD[a]*		*mg/day ± 1 SD[a]*		*mg/day ± 1 SD[a]*	
2	389	400	795 ± 80	955 ± 94[c]	184 ± 57	226 ± 31	978 ± 126	1180 ± 108[c]	589 ± 126	781 ± 108[c]
5	406	409	1471 ± 88	1543 ± 98	276 ± 125	324 ± 47	1746 ± 175	1867 ± 143	1340 ± 175	1458 ± 143
7	392	401	1169 ± 189	1188 ± 207	216 ± 58	164 ± 21	1382 ± 240	1352 ± 215	990 ± 240	951 ± 215
8	390	400	1072 ± 169	1151 ± 163	187 ± 59	225 ± 67	1259 ± 202	1376 ± 199	896 ± 202	976 ± 199
P (group)[d]	NS		NS		NS		NS		NS	

[a] Standard deviation of six determinations.
[b] S, saturated diet; P, polyunsaturated diet.
[c] P significantly greater than S at $P < 0.01$; in all other comparisons P was not significantly greater than S.
[d] Paired *t* test.

LDL-cholesterol accounted for only 67% of the decrement in total plasma cholesterol. These findings are in accord with the concept that polyunsaturated fats have multiple actions, whose expression may vary from patient to patient.

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