Effects of Dietary Fats on Plasma Lipids and Lipoproteins: an Hypothesis for the Lipid-Lowering Effect of Unsaturated Fatty Acids

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ABSTRACT Several aspects of the effects of dietary fat on plasma lipids and lipoproteins were investigated in 12 subjects during the long-term feeding of formulas containing 40% of their calories as either saturated or unsaturated fats. The changes in fatty acid composition of plasma lipids, shown previously to occur after prolonged feedings of a dietary fat, required 10–14 days to be complete and were synchronous with the effect of the fat on plasma lipid concentrations. The change in lipid concentration occurred in low but not in high density lipoproteins. The effects on lipid levels of the low density lipoproteins were found to occur with little or no effect on the concentration of the protein moiety of these lipoproteins; as a result, cholesterol- and phospholipid to protein ratios in low density lipoproteins fell during unsaturated fat feeding. The effects of dietary fat on plasma phospholipids were studied in detail: the relative amounts of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and lysophosphatidylcholine were unaffected by the type of dietary fat. However, the molecular species of phosphatidylcholine were markedly affected. More than 90% of the fatty acids at the α-position were saturated during both saturated and unsaturated feedings. In contrast, during unsaturated feedings, linoleate at the β-position outnumbered oleate by approximately 4:1, whereas during saturated feedings these two types of fatty acids were present in nearly equal amounts.

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This paper also presents the following hypothesis for the lipid-lowering effect of unsaturated dietary fat: since unsaturated fatty acids occupy a greater area than saturated acids, they alter the spatial configuration of the lipids into which they are incorporated; as a result, fewer lipid molecules can be accommodated by the apoprotein of the low-density lipoproteins (LDL), and thus the lipid content of the lipoprotein is lowered. The experimental findings of this study, while not proving this hypothesis, are consistent with it.

INTRODUCTION

It is well established that the concentration of cholesterol and phospholipid in the plasma is affected by the type of fat in the diet. Although nonglyceride components of dietary fats (such as cholesterol and plant sterols) can play a role in changing plasma lipid levels (2, 3), experiments utilizing synthetic oils containing glycerides alone, indicate that the glyceride per se will produce the observed effect (4). Several investigators have demonstrated that the lipid-lowering effect of these dietary oils can be related to the degree of polyunsaturation of their constituent fatty acids (5, 6). Most of the studies directed at determining the mechanism of this effect of unsaturated fats have been attempts to determine whether these fats lower plasma lipids by increasing fecal excretion of cholesterol and/or its metabolic products. Although several investigators (7–9) have found an increase in excretion of neutral and/or acidic sterols during unsaturated fat intake, others have demonstrated that such an effect is neither a necessary nor frequent accompaniment of the change in plasma cholesterol (4, 10, 11). Since there is no evidence that

TABLE I

Dietary Oils and Plasma Lipid Concentrations in 12 Patients

Patient	Age	Sex	Wt	Dietary oils		Cholesterol concentration‡		Triglyceride concentration‡	
				Saturated*	Unsaturated*	Saturated*	Unsaturated*	Saturated*	Unsaturated*
			kg						
1. M. W.	70	F	43	Coconut (21)	Safflower (21)	210 ±13	145 ±18§	130 ±16	68 ±11§
2. F. S.	61	F	58	Coconut (28)	Safflower (21)	265 ±19	200 ±12§	115 ±12	103 ±9
3. V. F.	38	F	51	Coconut (28)	Corn (28)	400 ±19	352 ±24§	56 ±11	55 ±9
4. I. G.	52	M	71	Coconut (28)	Safflower (21)	390 ±10	225 ±19§	–	–
5. J. Ba.	40	M	80	Butter (28)	Corn (28)	255 ±16	193 ±12§	199 ±12	162 ±6§
6. N. R.	36	F	67	Butter (136)	Safflower (55)	236 ±10	141 ±6§	201 ±11	111 ±26§
7. J. Bo.	38	M	75	Butter (28)	Trilinolein (42)	371 ±13	216 ±9§	205 ±22	156 ±17§
8. J. C.	39	M	65	Butter (126)	Safflower (70)	279 ±19	180 ±4§	201 ±24	149 ±13§
9. A. M.	21	F	62	Butter (28)	Corn (49)	429 ±14	391 ±5§	44 ±12	46 ±14
10. H. T.	57	M	41	Butter (42)	Corn (84)	339 ±17	253 ±7§	236 ±15	165 ±15§
11. B. K.	53	F	40	Butter (112)	Safflower (56)	304 ±23	233 ±12§	129 ±27	117 ±20
12. A. K.	44	M	76	Butter (56)	Corn (70)	270 ±14	178 ±11§	284 ±15	218 ±39§

* Saturated = during saturated fat feeding. Unsaturated = during unsaturated fat feeding. Figure in parentheses is duration of feeding in days.

‡ Based on last five bi-weekly values during each feeding period. Values represented are mean ±SD.

§ Concentration significantly lower during unsaturated feeding.

unsaturated fat inhibits cholesterol synthesis, this lack of consistent effect on excretion has given rise to the hypothesis that a decrease in plasma cholesterol content can reflect a shift of cholesterol from the plasma to other body pools (4, 12). An increase in fecal steroids, when present, may then be secondary to this redistribution, rather than the primary cause of the change in plasma cholesterol content.

If the lipid-lowering effect of unsaturated fats is not mediated primarily by an effect on fecal steroid excretion, it is possible that it results from alterations in plasma lipoproteins that are produced when unsaturated replace saturated fatty acids in circulating lipids. In order to acquire information that may be pertinent to this question, we have examined several aspects of the chemistry of lipids and the relationship of lipids to proteins in the plasma obtained from humans during the intake of highly saturated and unsaturated dietary fats. In addition to the results of these studies, we present an hypothesis in which the lipid-lowering effect of unsaturated fatty acids is related to the effect of their spatial configuration on lipid/protein associations in lipoproteins.

METHODS

Experimental subjects. Table I lists the 12 patients in whom the studies were carried out. Patients 1–4 were hospitalized at the metabolic unit of the Second (Cornell) Medical Division at Bellevue Hospital and patients 5–12 at the Hospital of The Rockefeller University. All were maintained on liquid formula feeding with the dietary oil comprising 40% of calories (13); the caloric intakes were adjusted to prevent weight changes. As indicated in Table I, these subjects included individuals with both normal and abnormal concentrations of plasma cholesterol, and all except patients 3 and 11 had occlusive vascular disease. Although in some patients hypertriglyceridemia of moderate degree had been noted in the past, at the time of the study the plasma triglyceride concentration was below 285 mg/100 ml in all subjects on both diets. Plasma lipoprotein electrophoresis by the method of Hatch and Lees (14) demonstrated that in those subjects with mild elevation of triglyceride the increase was endogenous (prebeta) in type. Those with hypercholesterolemia (subjects 3, 4, 7, and 9–11) had increased amounts of β-lipoprotein (type II, classification of Fredrickson, Levy, and Lees) (15).

Dietary oils. Table II lists the fatty acid composition of the dietary oils used in this study. Patients 6, 8, and 11 were participants in a separate study of the effect of dietary fat on sterol balance (10); they ingested formulas containing the natural oils to which cholesterol and plant sterol had been added so that the intake of these sterols was the same during saturated and unsaturated feedings. Subject 7 was

TABLE II

*Fatty Acid Composition of Dietary Oils**

	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Saturated								
Coconut	39.4	27.8	13.6		2.6	12.7	4.6	
Butter	4.2	15.0	36.4	3.6	13.3	27.4		
Unsaturated								
Corn			10.7		1.4	28.7	59.0	
Safflower			7.2		2.3	13.2	76.0	0.7
Trilinolein						4.2	95.8	

* Omitting fatty acids with chain length less than 12.

given the synthetic fat trilinolein;[1] in all other instances commercially available oils were used.

Plasma lipids. Bloods were obtained after an overnight fast. Disodium ethylenediamine-tetra-acetate (NaEDTA) was used as anticoagulant (approximately 1 mg/ml blood), and the plasmas were stored at 4°C for up to 10 days before analyses were completed. In those specimens in which fatty acid analyses or qualitative determination of phospholipids were to be done, a portion of the plasma was extracted within several hours and stored under nitrogen at −10°C. Lipoprotein separation was begun within 24 hr of blood drawing. Except for the data presented in Fig. 2, analyses were performed on plasma specimens obtained 10 days or more after the institution of any given dietary fat, at which time the cholesterol concentration had reached a plateau.

Plasma cholesterol was determined either by the method of Abell, Levy, Brodie, and Kendall (16) or by a semiautomated method (14); triglyceride either by the method of Van Handel (17) or by a semiautomated fluorometric method (14). In any one subject, a single method was used throughout the study. Lipid phosphorus was determined by the method of Stewart and Hendry (18) and protein by the method of Lowry, Rosebrough, Farr, and Randall (19). Plasma lipids were extracted with chloroform: methanol, 2:1, and lipid class separations were carried out by preparative thin-layer chromatography (TLC) (20). Methyl esters were prepared from cholesterol ester, triglyceride, and phospholipid after hydrolysis, by the use of boron trifluoride-methanol (21). Gas-liquid chromatography (GLC) was carried out as previously described (22).

Ultracentrifugal separation of plasma lipoprotein fractions was carried out as described by Havel, Eder, and Bragdon (23). The low density lipoprotein fraction (either d = 1.006–1.063 or d = 1.019–1.063) was centrifuged a second time at 1.063 and then dialyzed for at least 12 hr at 4°C in a nitrogen-saturated solution of 0.01% NaEDTA in 0.85% sodium chloride. This fraction was shown to contain uncontaminated β-lipoprotein when examined by immunoelectrophoresis.[2]

Plasma phospholipids were separated into phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, and lysophosphatidylcholine by TLC (24). Lipid phosphorus was determined for each fraction after identification with aqueous Rhodamine-G and elution from the silica gel with methanol. Recovery of phosphorus from the TLC plates varied from 87–95%.

Determination of the molecular species of the lecithin fraction was done essentially as described by Van Golde, Tomasi, and Van Deenen (25). The incubation mixture for the conversion of lecithin to diglyceride consisted of 1 mg of phospholipase C (Clostridium welchii, type 1 toxin[3]) dissolved in 1.5 ml 0.05 M Tris buffer, pH 7.2, and 0.5 ml 0.04 M calcium chloride to which the lecithin was added dissolved in 3-ml peroxide-free ether. The reaction was carried out at room temperature in an atmosphere of nitrogen with continuous agitation and was complete in 1 hr; the ether phase was dried over anhydrous sodium sulphate. The 1,2-diglyceride fraction, shown to be free of 1,3-diglyceride by TLC, was subfractionated by TLC with 12% $AgNO_3$ in Silica Gel H in a paper-lined tank kept at 4°C, the solvent system being 8% ethanol in chloroform. Diglyceride fractions were identified by Rhodamine G and immediately eluted with ether. Aliquots were used for saponification, methylation, and determination of fatty acid constituents after pancreatic lipase hydrolysis. The composition of the β-fatty acids of lecithin were determined after incubation of lecithin with phospholipase A (26), and recovery of the β-fatty acid and lysolecithin by TLC. The composition of the α- and β-fatty acids of the diglyceride fractions were determined as described by Mattson and Volpenheim (27).

[1] Kindly prepared by Dr. F. Mattson of Procter & Gamble Co., Cincinnati, Ohio.

[2] Kindly carried out by Dr. Robert S. Lees.

[3] Sigma Chemical Co., St. Louis, Mo.

RESULTS

Effect of dietary fats on plasma lipid concentrations. As indicated in Table I, plasma cholesterol concentration was lower during unsaturated than saturated fat feeding in all subjects. The difference averaged 27% of the value during saturated feeding. Changes in plasma phospholipids closely paralleled those in cholesterol in the six subjects in whom this determination was regularly carried out. In 7 of 11 subjects, triglyceride concentration was significantly lower during unsaturated than saturated period. In general, the triglyceride concentration was least affected in those subjects with the lowest initial values.

Comparison between effects of dietary fat on plasma lipid concentrations and fatty acid compositions. In patients 1–4 the time-course of the change in fatty acid composition of the individual esterified plasma lipids was compared with the change in the concentration of each plasma lipid class after exchanges of dietary fats (Fig. 1).

The major change in fatty acid composition involved linoleate (18:2) and oleate (18:1), which varied reciprocally. The time-course of the effect of the change in dietary oil on fatty acid composition, particularly of the phospholipid and cholesterol ester fractions, closely paralleled the changes in lipid concentrations in every case. In some instances the effect on the triglyceride fatty acids was completed more rapidly than in the case of phospholipid or cholesterol ester fatty acids.

The limits within which fatty acid compositions changed were similar in all four subjects. For cholesterol ester, the per cent of 18:2 never fell below 35–40%, even when saturated oils containing essentially no 18:2 comprised the sole dietary fat. The minimal value for phospholipid 18:2 approximated 15%, and for triglyceride 5–10%. During highly unsaturated feedings, 18:2 rose to about 70% in cholesterol ester, to 35% in phospholipids and to 40–50% in triglycerides.

Effects of dietary fat on the cholesterol and phospholipid content of high density lipoproteins. Table III lists the average value of three to six determinations of cholesterol concentration of d > 1.063 lipoproteins (HDL) from eight patients during saturated and unsaturated fat feedings. On saturated fats the mean value for the group was 32.6 ±2.0 compared with 32.4 ±2.4 mg/100 ml on unsaturated fats. HDL phospholipid con-

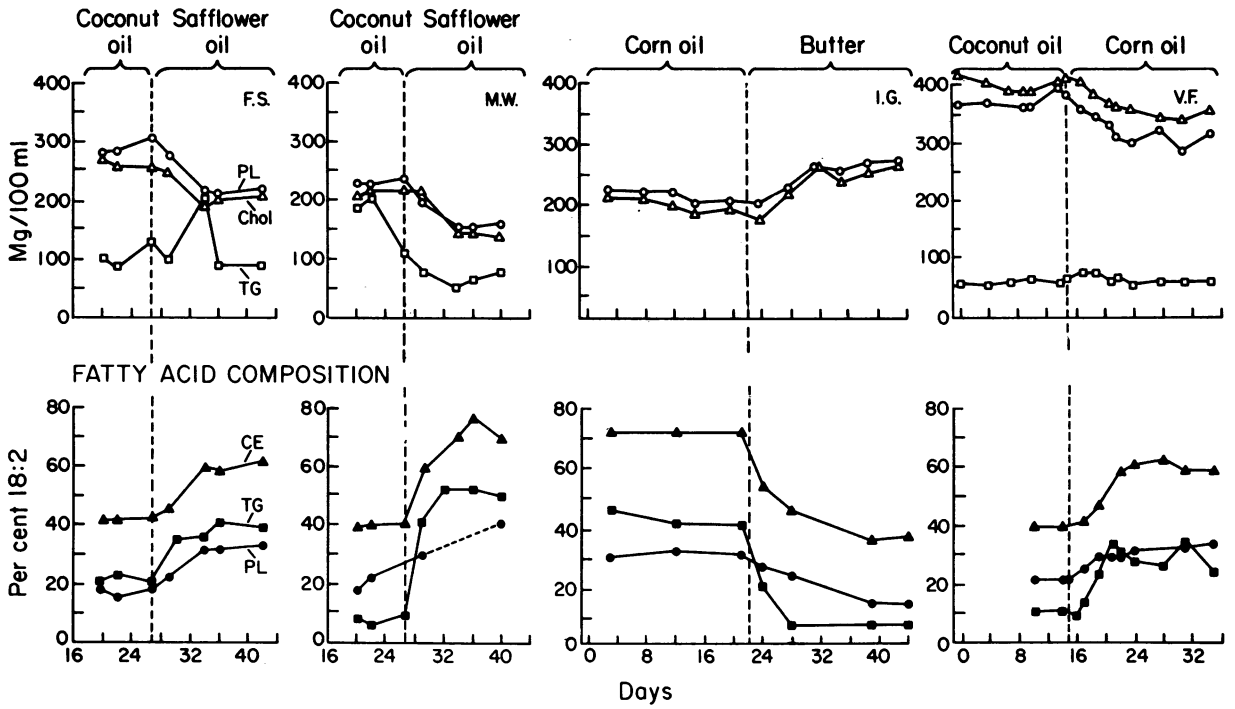

FIGURE 1 Comparisons between the time course of effects of changes in dietary fat on plasma lipid concentrations and fatty acids of esterified lipids.

centration also was unaffected by diet, with a mean of 62.0 ±4.7 during saturated and 60.4 ±5.9 mg/100 ml during unsaturated fat periods.

Effects of dietary fat on cholesterol, phospholipid, and protein content of purified low density lipoproteins (LDL). In contrast to the lack of effect of dietary fats on HDL, LDL cholesterol and phospholipid contents were regularly lower during unsaturated fat feedings (cholesterol 24%; phospholipid 26%). Yet, the protein content of the LDL was not significantly affected, the average value during unsaturated fat feeding being only 8.5% lower than in the saturated fat period. This re-

TABLE III

Effect of Dietary Fat on Components of High Density (HDL) and Low Density (LDL) Lipoproteins

Patient No.	HDL*				LDL‡									
	Cholesterol		Phospholipid		Protein		Cholesterol		Phospholipid		Cholesterol/protein		Phospholipid/protein	
Diet.....	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.
	mg/100 ml		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*ratio*		*ratio*	
2	37	40	69	77	107	108	160	121	–	–	1.50	1.12	–	–
5	40	25	48	37	109	102	147	114	103	73	1.35	1.12	0.94	0.72
6	31	33	66	66	94	86	117	94	110	80	1.24	1.09	1.17	0.93
7	29	31	46	42	152	128	206	135	154	111	1.36	1.05	1.01	0.87
8	23	26	58	63	114	104	168	114	114	82	1.47	1.10	1.00	0.79
9‖	28	25	–	–	231	246	400	362	236	184	1.73	1.47	1.02	0.75
10	37	39	81	76	161	169	260	195	167	140	1.61	1.15	1.04	0.83
11	36	40	66	62	216	180	276	210	231	164	1.28	1.17	1.07	0.91
11‖	–	–	–	–	196	160	260	194	202	134	1.33	1.21	1.04	0.84
Mean	32.6	32.4	62.0	60.4	153	140	222	171	164	121	1.43	1.16	1.04	0.83
±SE	±2.0	±2.4	±4.7	±5.9	±17	±15	±29	±28	±19	±15	±0.05	±0.04	±0.02	±0.03

* d > 1.063.
‡ d = 1.019–1.063.
§ See Table I.
‖ LDL – d = 1.006–1.063.

sulted in a change in cholesterol to protein ratios, from an average of 1.43 during saturated to 1.16 during unsaturated fat feeding, and in phospholipid to protein ratio from 1.04 to 0.83, respectively. Both changes were statistically highly significant.

In patients 4 and 9 the per cent of cholesterol in esterified form was determined during both dietary periods. Between 65 and 70% of total cholesterol was esterified in all four determinations; no effect of diet was evident.

Effect of dietary fat on the components of plasma phospholipids. Table IV lists the percent of composition of plasma phospholipid as phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (Sp), and phosphatidylethanolamine (PE) in patients 9 and 12. Although total phospholipid was 11 and 13% lower during unsaturated than during saturated fat feeding, the relative amounts of the four phospholipid subgroups were essentially unaffected by the diet change. As found by others (24), PC comprised approximately ⅔ of the total, and PC plus Sp exceeded 90% in all determinations. Phosphatidylinositol and lysophosphatidylethanolamine were less than 1% of the total during both types of feeding.

Effect of diet on the fatty acid composition of total plasma phospholipid. Table V lists the fatty acid composition of phospholipids isolated from plasma obtained from seven subjects during the two feeding periods. It is evident that while patient to patient variation was small, there were striking effects of diet change. The contents of saturated and tri- and tetra-enoic acids were essentially unaffected by diet; on the other hand, 18:1 and 18:2 were greatly altered, and the changes were reciprocal.

Effect of dietary fat on the molecular species of plasma lecithins. PC was isolated from the total phospholipid mixture, and the fatty acid compositions at the α- and β-positions were then determined. The first step was to convert the PC to diglyceride by reacting it with phospholipase C (28). As illustrated in Fig. 2, the resultant diglycerides were separated by TLC on silver nitrate–

TABLE IV

Effect of Dietary Fat on the Components of Plasma Phospholipids

Patient No.*	9		12	
Diet:	Sat.	Unsat.	Sat.	Unsat.
Plasma phospholipid, *mg/100 ml*	323	287	235	205
Phosphatidylcholine, %	61.8	58.5	71.0	71.0
Lysophosphatidylcholine, %	3.7	4.6	1.5	0.6
Sphingomyelin, %	32.4	34.7	25.0	25.8
Phosphatidylethanolamine, %	2.1	2.2	2.6	2.6

* See Table I.

TABLE V

Effect of Diet on Plasma Phospholipid Fatty Acids

Patient No.*	Phospholipid fatty acid: Saturated Sat.§	Saturated Unsat.§	18:1 Sat.§	18:1 Unsat.§	18:2 Sat.§	18:2 Unsat.§	Poly (20:3 and 20:4) Sat.§	Poly (20:3 and 20:4) Unsat.§
	%		%		%		%	
1	48	41	22	10	14	38	16	11
5	48	42	23	11	15	34	14	14
6	47	44	24	12	13	30	16	14
7	44	47	28	10	11	30	16	13
9‡	50	48	20	9	14	33	16	10
10	49	43	20	10	16	29	16	18
10‡	51	48	22	9	15	29	13	13
12‡	47	41	22	9	19	37	12	13

* See Table I.

‡ Phosphatidylcholine fatty acids.

§ Dietary fat.

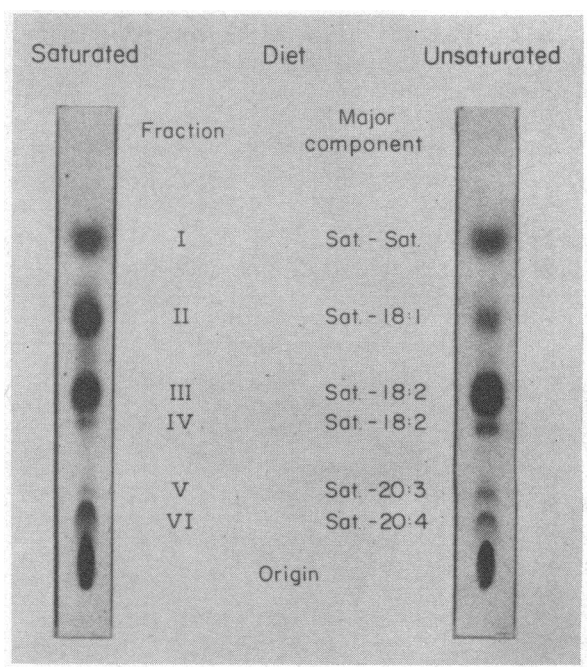

FIGURE 2 Thin-layer chromatograph of diglycerides derived from plasma lecithins during saturated and unsaturated feeding periods. The plate was made of 12% $AgNO_3$ in Silica Gel H and the solvent system was ethanol: chloroform, 8:92. For this illustration the chromatograph was stained by charring with 50% H_2SO_4.

TABLE VI

Composition of Diglycerides Derived from Plasma Lecithins during Saturated and Unsaturated Feedings

	Diet								Dominant fatty acids	
	Saturated				Unsaturated					
Fatty acid.....	Sat.*	18:1	18:2	Poly	Sat.*	18:1	18:2	Poly	α	β
Patient 9										
Fraction										
I	63 (94)‡	37	–	–	79 (92)	21	–	–	Sat.	Sat.
II	50 (86)	50	–	–	57 (91)	43	–	–	Sat.	18:1
III	47 (85)	8	45	–	46 (84)	5	49	–	Sat.	18:2
IV	–	–	–	–	48 (86)	6	46	–	Sat.	18:2
V	49 (92)	8	9	35§	48 (93)	8	–	45§	Sat.	20:3
VI	51 (88)	7	–	42‖	54 (92)	13	–	34‖	Sat.	20:4
Patient 12										
I	70	30	–	–	78	22	–	–	Sat.	Sat.
II	49	47	4	–	49	48	3	–	Sat.	18:1
III	44	4	52	–	43	4	53	–	Sat.	18:2
IV	–	–	–	–	39	6	56	–	Sat.	18:2
V	44	3	8	45§	48	4	3	46§	Sat.	20:3
VI	45	5	–	51‖	43	5	2	51‖	Sat.	20:4

* Sum of 14:0, 16:0, and 18:0.

‡ Figures given in parentheses in patient 9 are per cent of saturated fatty acid on α-carbon, determined as described in text.

§ 20:3.

‖ 20:4.

impregnated silicic acid. Six fractions could be isolated and the fatty acid composition of each was determined by GLC. Table VI lists the fatty acid compositions of these six fractions during saturated and unsaturated fat feedings in patients 9 and 12.

On both diets approximately half of the fatty acids of fractions II to VI were saturated, suggesting that the diglycerides contained one saturated and one unsaturated acid. Studies with pancreatic lipase (patient 9, Table VI) confirmed this and indicated that the saturated fatty acid was localized largely to the α-carbon in all fractions during both dietary periods. With this information, the structure of the diglyceride in each fraction could be determined from the GLC data. The cal-

TABLE VII

Effect of Dietary Fat on Per Cent of Composition of Molecular Species of Plasma Lecithins

	Patient:	9		10		12		Average	
Fraction*	Diet: Dominant fatty acids*	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.	Sat.	Unsat.
I	Sat.–Sat.	4.3	2.1	2.7	2.6	7.8	1.1	4.9	1.9
II	Sat.–18:1	30.0	12.0	30.9	14.8	25.9	11.2	28.9	12.7
III	Sat.–18:2	37.0	62.0	41.4	50.6	42.9	65.6	40.4	59.4
IV	Sat.–18:2	–	5.1	–	5.9	–	2.4	–	4.5
V	Sat.–20:3	14.6	8.8	8.8	12.8	9.8	9.3	11.1	10.3
VI	Sat.–20:4	14.1	9.6	16.2	13.9	13.6	10.4	14.6	11.3

* See Table VI and Fig. 3.

culations showed that fraction II was α-saturated, β-18:1; fractions III and IV were α-saturated, β-18:2 (we are unable to explain why this species separated into two subgroups on TLC); fraction V was predominantly α-saturated, β-20:3, and fraction VI was α-saturated, β-20:4. Fraction I consisted predominantly of di-saturated diglyceride, with 21–37% α-saturated, β-18:1.

Having illustrated in Table VI the types of diglyceride molecules derived from plasma PC, we then summarized the effect of dietary fat on the percent composition of these molecular species in three patients (Table VII). During saturated fat feeding periods, fractions I and II (containing all di-saturated and α-saturated, β-18:1 PC) constituted 34.3, 33.6, and 33.7% of the total, compared with 14.1, 17.4, and 12.3% during unsaturated fat feedings. Conversely, fractions III plus IV, containing the α-saturated, β-18:2 PC, were 37.0, 41.4, and 42.9% of the total during saturated, and rose to 67.1, 56.5, and 68.0% during unsaturated fat feedings. Fractions V plus VI, containing all of the α-saturated, β-20:3 and α-saturated, β-20:4 PC, were less strikingly affected by the exchange of dietary fats; they totaled 28.7, 25.0, and 23.4% during saturated, and 18.4, 26.7, and 19.7% during unsaturated fat feedings.

The marked effect of different dietary fats on the types of lecithin molecules in plasma is best seen by comparing the α-saturated, β-18:2 (fractions III plus IV) to the α-saturated, β-saturated plus β-18:1 (fractions I plus II). The ratio between these two types of lecithin molecules averaged 1.2 on saturated fat, and rose to 4.4 on unsaturated fat feedings.

DISCUSSION

The findings in this study provide evidence that, besides lowering the lipid content of plasma, the ingestion of polyunsaturated fats leads to considerable change in the composition of plasma lipids and lipoproteins.

It is well documented (29, 30) that a single fat meal alters the fatty acid composition of chylomicron triglyceride, such that they become more like that of the dietary fat. On the other hand, the lipids of the lipoproteins in normal fasting plasma remain unaltered after a single meal, and approach the fatty acid composition of the dietary fat only after continuous feeding for several weeks or longer (31, 32). In this study, we have demonstrated that in fasting plasma the changes in esterified fatty acids produced by diet are completed in 10–14 days and, within the limits of the analytical techniques, generally parallel the decrease in plasma phospholipid and cholesterol concentrations. This finding permits the possibility that the lipid-lowering effect of polyunsaturated fats may be a direct result of the incorporation of the dietary fatty acids into the lipids of LDL.

Whereas the effects of dietary fat on plasma lipid concentrations and fatty acid composition have been reported frequently, less information is available that defines this effect in terms of circulating lipoproteins. Although it is evident from previous studies that the cholesterol of LDL is decreased by ingestion of unsaturated fat, some investigators have (33) and others have not (34) found that a similar effect is produced in HDL. We examined this question in eight subjects and found no change in either cholesterol or phospholipid in that lipoprotein class, suggesting that the effect of unsaturated fat is a phenomenon limited to LDL.

In an effort to obtain more information about the details of this effect on LDL, we compared the change in phospholipid and cholesterol concentrations with that in the protein moiety of the d 1.019–1.063 lipoproteins. Whereas the change in LDL lipid occurred without effect on protein in most instances, in patients 7 and 11 a 16–17% fall in LDL protein occurred during unsaturated feeding; this decrease was certainly smaller than the 34 and 24% change in cholesterol and 28 and 29% in phospholipid in these two patients, and suggests a combined effect: both a fall in the lipid per lipoprotein as well as a decrease in the number of lipoprotein molecules.

The fact that LDL with differing protein content was observed under different dietary conditions in the present study is consistent with the findings of Oncley, Walton, and Cornwell (35). They showed that the Sf 3–8 lipoproteins could be divided into at least three fractions of increasing specific density (from 1.027 to 1.047) with protein content ranging from 18 to 24%; this range for protein content is comparable to that seen for LDL in the present study during saturated and unsaturated fat feedings, respectively, if we assume that triglyceride comprises 10% of the lipoprotein lipid (35). Thus, the lipoproteins of the LDL or Sf 3–8 fraction represent a spectrum of macromolecules with differing protein contents, and it is possible that variation in lipid:protein ratios within this fraction is determined, at least in part, by the fatty acid composition of the dietary fat.

Although these findings do not provide an explanation for the lipid-lowering effects of unsaturated fat, they are consistent with an hypothesis that relates this effect to the differences between the configuration of saturated and unsaturated fatty acids (Fig. 3). We propose this hypothesis: since unsaturated fatty acids occupy a greater area than saturated acids, they alter the spatial configuration of the lipids into which they are incorporated; as a result, fewer lipid moecules can be accommodated by the apoprotein of the LDL, and thus the lipid content of the lipoprotein is lowered.

Demel, Van Deenen and Pethica (36) have defined, in a monolayer of lecithin molecules, an in vitro model for a system in which the number of lipid molecules that

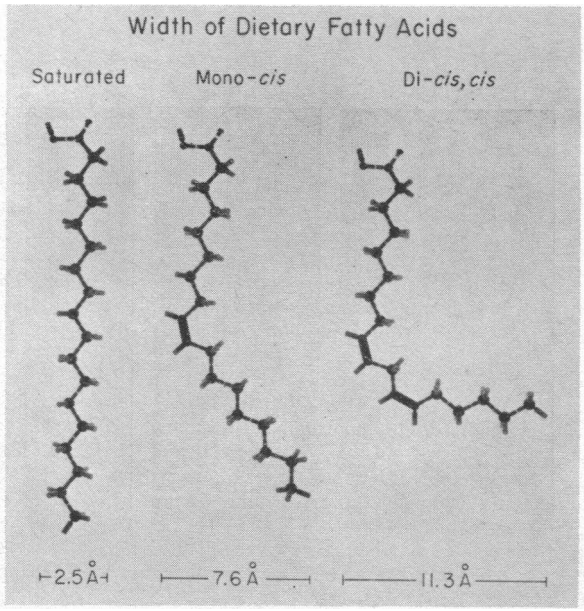

FIGURE 3 Scale models of the carbon skeleton of stearic, oleic, and linoleic acids.[4] These models illustrate the marked effect of *cis* double bonds on the configuration of these acids.

can be accommodated within a given area is determined by the fatty acid composition of the lipid. It is obvious that lipid/protein interaction in lipoproteins must be far more complex than the molecular interaction in a monolayer film. Yet it is possible that the arrangement is such that differences in configuration of the lipid molecules produced by substitution of unsaturated for saturated acids could be one of the factors determining the number of available sites for attachment to the apoprotein. On the assumption that lecithin is the lipid most closely associated with protein in LDL, we examined in detail the changes in the molecular species of lecithin molecules produced by substitution of saturated by unsaturated dietary fats. We found that the molecules shown to be larger by Demel et al. (36)–α-saturated, β-18:2–exceeded those with 18:1 at the β-position by about 4:1 during unsaturated feedings while the two types were present in nearly equal amounts during saturated feedings. This finding is compatible with the idea that the lower lipid/protein ratio seen during unsaturated feedings reflects decreased carrying capacity for lecithin on the apoprotein of LDL.

While this study provides several findings that are in accord with the hypothesis we propose, there are data in apparent contradiction to it and a conclusive test of its validity requires a knowledge of lipoprotein structure that is not presently available. One apparent contradiction arises from the finding that the fatty acids of the lipids in HDL are altered by diet just as in the case of LDL, yet unsaturated fat does not lower the concentration of HDL cholesterol and phospholipid. Although HDL protein has not been quantified during saturated and unsaturated fat feedings, let us assume that, since the lipid contents of HDL are unaltered, there has been no change in their lipid/protein ratio. Why, then, should the lipid capacity of HDL be governed by considerations different than for LDL? Marked differences in lipid composition between the two types of lipoproteins indicate that there are important differences in their structure that could account for the differences in response to dietary fat exchange. Perhaps most important for present consideration is the much lower lipid to protein ratio in HDL. It is conceivable that the number of lipid molecules that can be accommodated by an apoprotein will be determined by the configuration of the lipid only when the carrying capacity of the apoprotein is at or near saturation, the situation that probably is present in LDL, but which may not be in HDL. Meaningful consideration of this line of reasoning must await further knowledge of the details of structure of both types of lipoproteins.

The effects of drugs and low-fat diets on lipid/protein ratios in lipoproteins have not yet been evaluated. If the change in lipid/protein ratio in LDL found in this study is unique, it would provide further support for the proposed hypothesis. If, on the other hand, changes in this ratio generally parallel lowering of plasma lipids, one must assume either that configurational effects are not critical in the lipid-lowering effect of unsaturated fat, or that this is only one of several mechanisms by which the amount of lipid transported by LDL apoprotein can be altered. Further investigations designed to answer the questions raised by these considerations could provide information concerning the mechanisms of lipid-lowering procedures as well as to the nature of lipid/protein associations in lipoproteins.

[4] Dimensions refer to measurements of width from scale molecular models and are not intended to reflect the measurement of the fatty acids in situ.

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