Effects of Polyunsaturated Fats on Lipid Metabolism in Patients with Hypertriglyceridemia

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ABSTRACT Studies were carried out on the effects of polyunsaturated fats on lipid metabolism in 11 patients with hypertriglyceridemia. During cholesterol balance studies performed in eight patients, the feeding of polyunsaturated fats, as compared with saturated fats, caused an increased excretion of endogenous neutral steroids, acidic steroids, or both in most patients. Increases in steroid excretions were marked in some patients and generally exceeded the decrement of cholesterol in the plasma compartment. The finding of a greater excretion of fecal steroids on polyunsaturated fats in hypertriglyceridemic patients contrasts to the lack of change in sterol balance previously reported for patients with familial hypercholesterolemia; however, other workers have found that polyunsaturated fats also enhance steroid excretion in normal subjects.

In most of the patients, simultaneous studies were carried out on biliary lipid composition, hourly outputs of biliary lipids, and pool sizes of bile acids. In several but not all patients, fasting gallbladder bile became more lithogenic after institution of polyunsaturated fats. This increased lithogenicity was not due to a decrease in bile acid pools; in no case was the pool decreased by polyunsaturated fats. On the other hand, two patients showed an increased output of biliary cholesterol, and frequently there was an increase in fecal neutral steroids that were derived from cholesterol; thus, polyunsaturated fats may increase bile lithogenicity in some patients through mobilization of cholesterol into bile.

Reductions in plasma cholesterol during the feeding of polyunsaturated fats was seen in most patients, and these changes were usually associated with a decrease in concentration of plasma triglycerides. In fact, the degree of cholesterol lowering was closely correlated with the extent of triglyceride reduction. Therefore, in hypertriglyceridemic patients polyunsaturated fats may contribute to cholesterol reduction by changing the metabolism of triglycerides or very low density lipoproteins.

The findings of changes in the metabolism of cholesterol, bile acids, and triglycerides in the patients of this study suggests that polyunsaturated fats may cause a lowering of cholesterol through multiple mechanisms, and it seems unlikely that a single action can explain all the effects of these fats on the plasma lipids.

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INTRODUCTION

During the past two decades, since the discovery that polyunsaturated fats cause a decrease in plasma cholesterol concentrations, there has been increasing interest in the possible use of these fats in the prevention of atherosclerosis. In fact, evidence has recently been obtained from large clinical trials to suggest that polyunsaturated fats may reduce the prevalence of clinical manifestations of atherosclerosis (1, 2). If the validity of this evidence can be strengthened by future trials, the dietary intake of these fats will undoubtedly become widespread.

Although lowering of plasma cholesterol during ingestion of polyunsaturated fats can readily be demonstrated in both normal and hyperlipidemic subjects, the mechanisms for this action have remained elusive. Since the question of how polyunsaturated fats produce a decrease in plasma cholesterol may be related to their long-term safety and even beneficial effects, numerous studies have been carried out to elucidate these mechanisms (3–28). Indeed, a variety of different effects have been reported, but investigations have thus far not provided a sufficient explanation for their

hypocholesterolemic action. Many findings have not been consistent from one laboratory to another, and these differences have generated considerable controversy regarding the actions of polyunsaturated fats. A possible explanation for some of the inconsistencies in human studies could be that different types of subjects may have fundamental differences in lipid metabolism, and the response of all subjects to dietary changes may not be identical. In the present study, this possibility has been investigated more thoroughly; effects of polyunsaturated fats on plasma lipids and cholesterol balance were examined in patients with hypertriglyceridemia, a group that previously has not been studied in detail.

The results show that exchange of polyunsaturated fats for saturated fats frequently causes an increased excretion of neutral and acidic steroids, and these increases may contribute to reduction of cholesterol in both plasma and tissue compartments. However, changes in sterol metabolism are probably not the only mechanisms for cholesterol lowering in hypertriglyceridemic patients; decreases in plasma cholesterol were found to be linked to reductions in triglyceride levels, and alterations in triglyceride metabolism may be yet another way in which polyunsaturated fats lead to a decline in plasma cholesterol.

METHODS

Patients. Studies were carried out in 11 male patients at the Veterans Administration Hospital, San Diego, Calif. The age, weight, percent of ideal weight, and diagnosis for each patient are given in Table I. All patients had increased levels of plasma triglycerides; nine patients had type IV lipoprotein patterns, and two (W. D. and R. F.) showed type V patterns (30). Three patients (J. E., D. W., and M. P.) had mild abnormalities in glucose tolerance as typical of patients with hypertriglyceridemia (31), but none required insulin or oral hypoglycemic agents. A genetic classification of the type of hypertriglyceridemia according to Goldstein, Hazzard, Schrott, Bierman, and Motulsky (32, 33) was not carried out. Two patients (W. V. and R. F.) had previous cholecystectomy for gallstones; routine oral cholecystograms were not performed on the remaining patients, but more had symptoms of cholelithiasis.

TABLE I
Clinical Data

Patient	Age	Weight	Ideal weight*	Diagnosis
	yr	*kg*	%	
N. B.	52	76	116	Hyperlipidemia (type IV)‡
M. P.	53	75	111	IHD,§ hyperlipidemia (type IV)
B. D.	56	85	116	IHD, hyperlipidemia (type IV)
E. McD.	63	75	118	IHD, hyperlipidemia (type IV)
A. T.	48	71	111	IHD, hyperlipidemia (type IV)
R. F.	51	95	133	Hyperlipidemia (type V)
D. W.	44	82	125	Hyperlipidemia (type IV)
W. V.	48	74	103	IHD, hyperlipidemia (type IV)
J. S.	50	113	159	Hyperlipidemia (type IV)
J. E.	50	66	98	IHD, hyperlipidemia (type IV)
W. D.	45	85	119	IHD, hyperlipidemia (type V)

* According to life insurance tables for height, weight, and sex (29).
‡ According to Fredrickson, Levy, and Lees (30).
§ IHD, ischemic heart disease.

Diets. During hospitalization, the patients were fed a diet of mixed solid food and formula containing 40% of calories as fat. The basic composition and pattern of this diet has been previously described in detail (34). The patients were given three formulas and two solid food meals per day; calories were divided approximately equally between the feedings. Formulas were given at 8:30 a.m., 1:00 p.m., and 8:30 p.m.; they contained 15% of calories as milk protein (RI-5, Ross Laboratories, Columbus, Ohio), 45% as dextrose, and 40% as fat (35). One solid food meal was given at 11:00 a.m., and it contained dry cereal (cornflakes), nonfat bread, skim milk, added fat, and sugar for coffee. At 4:00 p.m. the second meal was given which consisted of chicken that was stripped of fat, nonfat bread, potatoes, added fat, and carbonated beverage (cola). Fat comprised approximately 40% of calories in solid food meals. During the first period of study (period I) the fat was saturated in the form of lard; in period II, the saturated fats were replaced by polyunsaturated fat as safflower oil. Both periods were usually about 1 mo. Intakes of cholesterol ranged from 173 to 282 mg/day during period I and from 63 to 137 mg/day in period II. Vitamin and mineral supplements were given daily. Each patient was weighed daily, and caloric intake was adjusted to maintain total body weight at a constant level throughout the study.

Cholesterol balance studies were carried out on eight patients according to methods described previously (36–39). Stools were collected throughout both dietary periods and were usually combined into 4- or 5-day pools.

Fecal neutral and acidic steroids were isolated separately, and their masses were determined by gas-liquid chromatography (GLC). GLC analysis of neutral steroids distinguished between plant sterols and cholesterol and between the two families of bacterial conversion products derived from plant sterols and cholesterol during intestinal transit (5αH, 3βOH, and 5αH, 3-keto compounds). Analyses were carried out entirely by chemical procedures. To correct for losses of neutral steroids, β-sitosterol was given in small doses either in the form of capsules or inherent in the oil (38), and excretions of acidic steroids were corrected for variations in fecal flow by use of chromic oxide (39).

Plasma lipids. Measurements of concentrations of plasma cholesterol and triglycerides were carried out weekly or biweekly; analyses were performed on a Technicon Auto-Analyzer (model II, Technicon Instruments Corp., Tarrytown, N. Y.) (40, 41). Lipoprotein electrophoresis was carried out according to the method of Noble (42).

Lipid composition of gallbladder bile. In eight patients, samples of fasting gallbladder bile were obtained two or three times during each dietary period for analysis of cholesterol, bile acids, and phospholipids. Samples were aspirated from a single lumen tube positioned by X-ray guidance in the second portion of the duodenum. Gallbladder contraction was stimulated by intraduodenal in-

jection of a liquid formula containing 40% of calories as fat; the formula was free of cholesterol and phospholipids. Gallbladder bile was then collected by siphonage over a period of 20 min. The collected bile (30–50 ml) was thoroughly mixed and a 10-ml sample was retained for analysis; the remainder was returned to the patient via the tube. Samples were added immediately to 30-ml chloroform-methanol (2:1). Cholesterol and phospholipids were partitioned into the chloroform phase, and conjugated bile acids were in the water-methanol phase (43, 44). The phases were separated, and appropriate washings were carried out for quantitative isolation of individual constituents. After evaporation of the chloroform phase, cholesterol was measured directly on GLC as the trimethylsilyl (TMS)[1] ether (37, 45); 5α-cholestane was used as an internal standard for GLC. Phospholipids were measured by the method of Rouser, Sidney, and Akira (46), and bile acids were determined by a standard enzymatic procedure (47, 48). Results were expressed as a mole percent for each lipid component according to Admirand and Small (48). Since previous studies have shown that bile from gallstone patients frequently contains a greater amount of cholesterol than can be solubilized by bile acids and phospholipids present (48, 49), the term "lithogenic bile" has been employed for bile that is supersaturated with cholesterol. However, there is disagreement as to the precise limits of cholesterol solubility in bile (48–53), and in the present paper, the term "lithogenic" will be used in a comparative sense. Bile samples with a lower molar percentage of cholesterol are said to be less lithogenic than samples with a higher percentage; no attempt has been made to demarcate between lithogenic and nonlithogenic bile.

Outputs of biliary lipids. Hourly outputs of biliary cholesterol, bile acids, and phospholipids during constant feeding of a formula diet were determined in six patients by the marker dilution technique of Grundy and Metzger (54). After an overnight fast, a 3-lm tube was positioned in the duodenum with the two most proximal outlets adjacent to the ampulla of Vater and the third outlet 10 cm distally. The tube was placed in the correct position with X-ray guidance. A liquid formula containing 40% fat was infused continuously through the one proximal lumen; β-sitosterol was also infused as a dilution marker. During period I lard was used as the fat, and safflower oil was used in period II. After allowing 4 h for gallbladder contraction and for stabilization of hepatic bile secretion, hourly samples were obtained for 6 h from the second proximal and distal outlets by slow and continuous aspiration. Less than 5% of intestinal contents passing these ports was removed. Since the input of β-sitosterol was known with precision, measurement of β-sitosterol and cholesterol recovery at the distal outlet gave the rate of cholesterol secretion. These data combined with measurements of concentrations of bile acids and phospholipids relative to cholesterol at the proximal outlet permitted calculation of the hourly outputs of bile acids and phospholipids. Equations used in these calculations have been presented previously along with corrections for cholesterol contents of formula diets (54).

Bile acid pool size. At the beginning of the formula infusion, 5 μCi of [24-^{14}C]cholic acid (New England Nuclear, Boston, Mass.) in 10-ml ethanol was flushed through the distal outlet with 100-ml water. During the 4 h after injection of the isotope, the radioactive bile acid was allowed to equilibrate with nonradioactive bile acids in the enterohepatic circulation. In the samples obtained for measurements of biliary lipids outputs, radioactivity was determined and expressed as counts per milligram of total bile acids. Fig. 1 shows ratios of counts to total bile acids ("specific activity") on hourly samples during the several hours of bile collection for both periods I and II. In most patients, specific activities of bile acids were relatively constant during the period of bile collection, and there was little or no tendency for a decline in activity towards the end of the period. Nevertheless, in some patients, specific activities fluctuated somewhat throughout the period, and measurement of multiple points was necessary to obtain a reliable average. From these data, the pool size of bile acids was calculated as follows:

Bile acid pool size (milligrams) = radioactivity injected (counts per minute) ÷ bile acid specific activity (counts per minute per milligram), where bile acid specific activity was the average hourly specific activity over the collection period.

In measurement of the pool size of total bile acids, the following assumptions were made: (*a*) that the isotope was completely mixed with bile acids in the enterohepatic circulation (EHC) during the 4-h equilibration period; (*b*) that significant quantities of the isotope were not lost from the EHC before complete mixing had occurred; (*c*) that [^{14}C]cholic acid and its conjugates were recycled at the same rate as other bile acids during the collection period; and (*d*) that new synthesis of bile acids during the collection period was small compared to the total pool size of bile acids. The relative constancy of specific activities during the several hours of bile collection suggests that complete equilibration had occurred and that bile acid synthesis during this period was insignificant. Values did not appear to fall during the latter part of the collection period as would have been expected if significant quantities of new bile acids had been synthesized. It should be emphasized that the results obtained by this measurement represents the size of the circulating pool of bile acids. If some bile acids had been retained in the gallbladder from the previous night's fast and did not enter the EHC throughout the entire study, they would not be included in the measurement of pool size. Also, bile acids that were sequestered in colonic contents and did not readily recycle in the EHC would not have been measured. Therefore, the value for pool size obtained by this method does not necessarily represent all bile acids present in the body and may not correspond exactly to that derived from the method of Lindstedt (55).

Individual bile acids. The relative proportions of the individual bile acid species present in the gallbladder were measured by GLC of TMS ethers of bile acid methyl esters. Analyses were carried out on 1% Hi-Eff 8BP columns (Applied Science Laboratories, Inc., State College, Pa.) as described previously (36).

RESULTS

Plasma lipids. Mean values for concentrations of plasma cholesterol and triglycerides during the two dietary periods are shown in Table II, and percent changes for these two lipids are compared in Fig. 2. In 7 of 11 patients, polyunsaturated fats caused a significant reduction in plasma triglyceride concentra-

[1] *Abbreviations used in this paper:* EHC, enterohepatic circulation; LDL, low density lipoprotein; TMS, trimethylsilyl; VLDL, very low density lipoprotein.

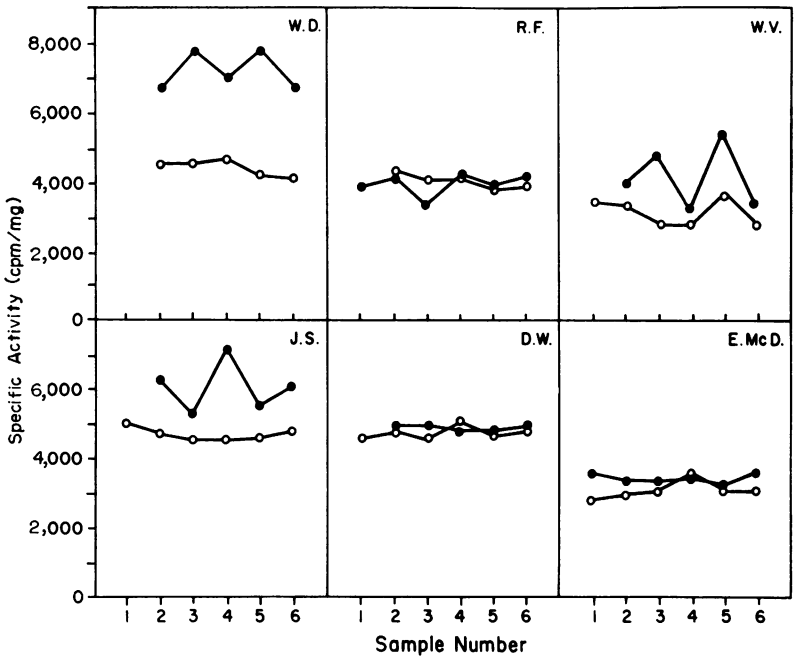

FIGURE 1 Specific activities of total bile acids during measurements of biliary lipid outputs in periods I and II. [24-^{14}C]Cholic acid was administered intraduodenally 4 h before sample 1. Continuous aspiration of duodenal contents was carried out for the subsequent 6 h, and aspirations were divided into hourly samples. Specific activities represents the ratios of radioactive counts to total bile acids. In some cases, only the last five samples were included because it appeared from the first value that equilibration had not occurred. Pool sizes of bile acids were calculated from the means of the five or six determinations. ●, period I; ○, period II.

tions. In general, the percentage reduction was greatest in those patients in whom initial levels of triglycerides were high. One patient (W. D.) is not shown in Fig. 2; he actually had a significant increase in plasma triglycerides on polyunsaturated fats, as shown in Table II. In this patient, however, chylomicrons were detected by electrophoresis throughout both dietary periods (type V pattern); therefore, it was not possible to rule out the possibility that unsaturated fats might have lowered endogenous triglycerides. Fig. 2 compares decreases in cholesterol concentrations with changes in plasma triglycerides. In most cases, the percent reduction in cholesterol was closely related to the extent of triglyceride reduction. Major decreases in cholesterol occurred only when triglycerides were markedly reduced. In those patients who failed to respond with significant triglyceride falls, changes in plasma cholesterol were also minor or absent.

Fecal steroid excretion. Table III shows excretions of neutral and acidic steroids as well as cholesterol balance for eight patients during exchange of polyunsaturated for saturated fats. Five of eight patients had significant increases in total neutral steroids on polyunsaturated fats; the average daily increment for these five patients ranged from 207 to 521 mg/day. Another patient (J. E.) showed an abrupt increase in output of neutral steroids in the last half of period II, and neutral steroids in the last 12 days of period II were significantly greater than in the whole of period I (605 ± 36 [$n=6$] vs. 764 ± 16 [$n=3$], [$P<0.05$]). In most patients, the increase in neutral steroids became apparent shortly after introduction of polyunsaturated fats and persisted throughout period II. Excretions of acidic steroids were numerically greater in seven of eight patients on polyunsaturated fat, but these increases were statistically significant in only four. In one patient (W. D.), the increment in acidic steroids in period II was marked (772 vs. 1,718 mg/day).

Values for fecal neutral steroids shown in Table III represent a mixture of endogenous neutral steroids and a small quantity of unabsorbed dietary cholesterol. Although dietary intakes of cholesterol were deliberately kept as low as possible, they were nevertheless somewhat greater in period I than in period II. Thus, in those patients who had an increased excretion of total neutral steroids in period II, actual differences between endogenous neutral steroids in the two periods were probably even greater than shown in Table III. For this reason, values for cholesterol balance are also given; cholesterol balance is defined here as the differ-

ence between total steroid output and cholesterol intake. In seven of the eight patients cholesterol balance was significantly more negative on polyunsaturated fats than on saturated fats.

In the first six patients shown in Table III, dietary intakes of β-sitosterol were somewhat higher on polyunsaturated fats than during the first period; this difference was due to β-sitosterol inherent in the safflower oil. This oil contains much less phytosterols than many other vegetable oils such as corn oil, and these six patients received an average increment of only 234 mg/day of β-sitosterol during period II. Previous studies have shown that large doses of β-sitosterol in the range of 10 g/day can significantly inhibit the absorption of cholesterol (but not bile acids) (56). It was therefore possible that the increases in neutral steroids observed in some of our patients could have been due in part to the slightly greater intakes of β-sitosterol in period II. However, in the three patients (W. D., W. V., and J. S.), in whom excretions of neutral steroids were significantly greater in period II, increments in fecal neutral steroids exceeded the enhancement of β-sitosterol intake. Since the differences in intakes of β-sitosterol between the two periods were relatively small and did not even exceed the changes in neutral steroid excretion, it is doubtful that these differences could account for the enhanced output of neutral steroids. Nevertheless, in the latter two patients (A. T. and R. F.) presented in Table III, greater care was taken to balance the β-sitosterol intakes of the two dietary periods; despite a uniform ingestion of β-sitosterol throughout the whole study, excretions of neutral steroids were still significantly higher on polyunsaturated fats for both patients.

The cholesterol balance studies showed that increases in neutral or acidic steroids or both fractions frequently occurred in our patients with hypertriglyceridemia. In several respects, however, the findings were variable from one patient to another. An increase did not occur in both steroid fractions in every patient during period II. Some patients had a greater excretion of neutral steroids only; in others, acidic steroids alone were increased; in several patients, both fractions were increased, but in a few neither steroid showed an enhanced output when considered by itself. When changes actually occurred, the magnitude of increases in the two steroid fractions were variable from patient to patient.

Since the response in steroid outputs to polyunsaturated fats seems to be variable, the relation between absolute changes in plasma cholesterol and fecal steroids should be examined. The maximum decrement of cholesterol in the plasma compartment for any patient of

TABLE II

Plasma Lipids during Exchange of Saturated and Unsaturated Fats

Patient	Period	Plasma cholesterol	Plasma triglyceride
		*mg/100 ml SD (n)**	
N. B.	I	211±29 (5)	1,194±147
	II	138±31 (5)	633±123
	Δ‡	−73 (35)§	−561(47)§
M. P.	I	252±12 (7)	477±139
	II	165±41 (4)	256±41
	Δ	−87 (35)§	−221 (46)§
B. D.	I	228±22 (5)	431±93
	II	156±4 (5)	262±24
	Δ	−72 (31)§	−169 (39)§
E. McD.	I	284±13 (6)	632±106
	II	214±26 (6)	397±117
	Δ	−70 (25)§	−235 (37)§
A. T.	I	210±10 (6)	249±51
	II	178±13 (4)	187±23
	Δ	−32 (15)§	−62 (25)§
R. F.	I	418±33 (8)	2,223±151
	II	352±24 (7)	1,710±120
	Δ	−66 (16)§	−513 (23)§
D. W.	I	171±11 (8)	256±21
	II	144±7 (8)	204±42
	Δ	−27 (14)	−47 (19)
W. V.	I	158±4 (8)	186±18
	II	136±10 (8)	160±15
	Δ	−22 (14)§	−26 (14)§
J. S.	I	263±16 (6)	411±129
	II	276±8 (6)	383±21
	Δ	+13 (−5)	−28 (7)
J. E.	I	229±9 (6)	295±27
	II	196±8 (6)	271±44
	Δ	−33 (14)	−24 (7)
W. D.	I	197±19 (80)	599±177 (7)
	II	174±13 (8)	821±229
	Δ	−23 (12)§	+222 (37)

* *n* = number of determinations (weekly or biweekly) of cholesterol and triglycerides. Duration of periods ranged from 17 to 38 days, as shown in Table III. In some patients, the first value of period II, when plasma lipid values were decreasing, was omitted in the comparison.

‡ Difference between periods I and II. The first number is the absolute change in mg/100 ml and the number in parentheses is percent change. The percent chage was calculated as the difference between periods I and II divided by period I times 100. A negative value indicates a decrease in period II.

§Values were significantly lower in period II than in period I (*P* 0.05 or less) (Student's *t* test).

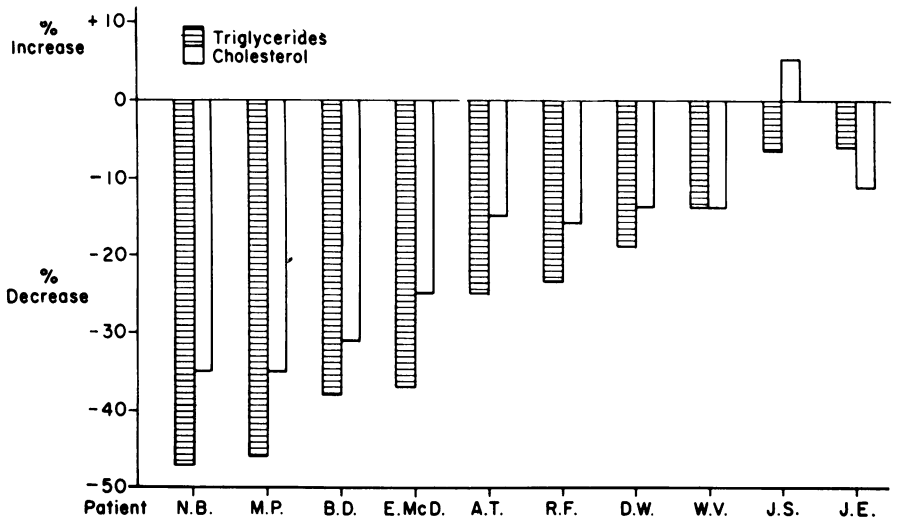

FIGURE 2 Percent changes for plasma triglycerides and cholesterol during period II. Mean values shown in Table II are compared. Results are presented for 10 patients. One patient (W. D.) is not shown; he had an increase in triglycerides in period II, but chylomicrons were detected by electrophoresis in both dietary periods.

this study was calculated to be 2.93 g.[2] The total increments in steroid output in period II, as compared to period I, are given in Table III. These increments ranged from 5.65 to 50.19 g for the whole of period II; in absolute quantities they consistently exceeded the maximum decrease in plasma cholesterol. Therefore, the increase in fecal steroids could not have been derived solely from cholesterol lost from the plasma compartment. Also, the degree of plasma cholesterol lowering was not closely correlated the magnitude of changes in fecal steroids.

The greater quantities of fecal steroids that were observed in most patients could have been derived from existing body pools or from newly synthesized cholesterol. The decrease in plasma cholesterol on polyunsaturated fats is compatible with a reduction in other pools, but the data also suggest that increased synthesis may have at least contributed to greater fecal excretion. In most patients who showed an increased excretion of steroids, the greater outputs persisted throughout the whole of period II, and such an increase for approximately 1 mo is suggestive that a new steady state with enhanced cholesterol synthesis may have occurred. The latter mechanism is dramatically illustrated in patient W. D. This patient had an increment in steroid excretion of approximately 50.1 g in period II. At the end of this period additional balance studies were carried out. During the next 30 days (period III), he continued to receive polyunsaturated fats, but he was also given clofibrate. In this period, neutral steroid excretion remained constant, but acidic steroid outputs decreased to some extent. Nevertheless, the total increment for period III, as compared to period I, was 31.1 g. Finally, in a fourth period of 31 days, clofibrate was discontinued and polyunsaturated fats were continued, as in period II. Again, increased excretion was maintained, and the increment for this period was 39.8 g. Throughout periods II, III, and IV, this patient excreted an excess of 121 g of steroids in comparison to period I when he was receiving saturated fats. This great excess of steroids must have been derived in large part from newly formed cholesterol.

Biliary lipids. The average composition of lipids (cholesterol, bile acids, and phospholipids) in gallbladder and hepatic bile are presented for the two dietary periods in Table IV. Institution of polyunsaturated fats had no consistent effects on lipid composition of gallbladder bile; four patients (M. P., J. S., J. E., and E. McD.) had increases in molar percent of cholesterol, and the remaining four had little change or decreases. During constant infusion of liquid formula diets, molar percent of cholesterol in hepatic bile generally did not change between the two periods; there was no increase in lithogenicity of hepatic bile on polyunsaturated fat.

In Table V, hourly outputs of biliary lipids are

[2] The method for estimating the decrement of cholesterol in the plasma compartment has been presented previously (14). The decrement (grams) is calculated as follows: plasma volume (liters) × average change in plasma cholesterol (period I–period II) (mg/100 ml) ÷ 10. The plasma volume is assumed to be 4.5% of total body weight (57). For those patients of the present study who showed a decrease in plasma cholesterol in period II, the total decrement ranged from 0.66 to 2.93 g.

TABLE III

Fecal Steroid Excretion during Exchange of Dietary Fats

Patient	Period	Days: no. determ.*	β-Sitosterol intake	Cholesterol intake	Total neutral steroids	Acidic steroids	Total fecal steroids	Cholesterol balance	Total increment (II-I)
			mg/day	*mg/day*	*mg/day±SD*				*g*
W. D.	I	30:6	346	282	859±78	722±249	1,581±232	1,299±232	
	II	30:6	606	126	1,380±151	1,718±406	3,098±505	2,972±505	
	Δ		260	156	521§	996§	1,517§	1,673§	50.19
	III	30:6	606	105	1,386±186	1,056±440	2,442±395	2,337±395	31.14
	IV	31:6	606	105	1,393±208	1,295±183	2,688±264	2,583±264	39.80
W. V.	I	30:6	346	214	621±88	255±34	876±104	662±104	
	II	38:8	582	98	882±90	337±68	1,219±86	1,121±86	
	Δ		236	116	261§	82§	343§	459§	17.44
J. S.	I	24:6	346	217	835±58	24±56	1,096±122	879±122	
	II	24:6	611	85	1,167±117	292±44	1,459±90	1,374±90	
	Δ		265	132	332§	31	363§	495§	11.88
E. McD.	I	28:7	346	217	677±97	270±82	947±128	730±128	
	II	21:7	582	122	740±100	381±58	1,121±119	999±119	
	Δ		236	95	63	111§	174§	269§	5.65
D. W.	I	30:6	346	231	867±100	239±85	1,106±141	875±141	
	II	35:7	571	121	953±108	322±97	1,275±174	1,154±174	
	Δ		225	110	86	83	169	279§	9.76
J. E.	I	23:6	346	173	605±36	444±49	1,049±46	876±46	
	II	24:6	532	63	677±20	502±108	1,179±142	1,166±142	
	Δ		185	110	62	58	130	240§	5.76
A. T.	I	17:6	346	200	688±269	366±68	1,054±83	854±83	
	II	18:6	364	125	895±123	496±79	1,391±158	1,266±158	
	Δ		18	75	207§	130§	337§	412§	7.42
R. F.	I	36:6	362	188	1,704±147	1,038±242	2,742±319	2,554±314	
	II	30:6	403	137	1,999±277	933±225	2,932±327	2,795±327	
	Δ		41	51	295§	−105	190	241	7.23

* Duration of balance period (days) and number of successive stool pools analyzed; the ratio of the two figures give the average number of days in each pool.

‡ Total increment in steroid excretion of period II compared to period I. This value was obtained by multiplying the difference between cholesterol balance for periods I and II times the number of days in period II.

§ Period II significantly different from period I (P 0.05 or less) (Student's t test).

|| Throughout periods III and IV, patient W. D. was continued on the unsaturated fat. In period III he received clofibrate (1 g twice daily), and in period IV the clofibrate was discontinued. The total increment in steroid excretion is obtained by comparison with period I.

presented for six patients along with pool sizes of bile acids. Two patients (W. D. and R. F.) showed significant increases in biliary outputs of cholesterol during period II; the remaining patients had no differences on the two diets. In most patients, no significant changes were found for outputs of bile acids

TABLE IV

Effects of Fat Exchange on Lipid Composition of Gallbladder and Hepatic Bile

Patient	Period	Gallbladder bile*			Hepatic bile‡		
		Cholesterol	Bile acids	Phospholipids	Cholesterol	Bile acids	Phospholipids
		molar %			*molar %*		
M. P.	I	5.4	75.4	19.3	—	—	—
	II	10.4	66.1	23.5	—	—	—
J. S.	I	2.8	72.5	23.6	7.7	59.4	33.7
	II	9.0	63.3	27.8	8.3	66.1	25.7
J. E.	I	6.8	73.2	21.7	—	—	—
	II	9.1	62.3	28.8	—	—	—
E. McD.	I	9.7	63.7	26.6	5.1	76.3	18.7
	II	12.8	42.0	45.2	4.3	74.6	21.0
N. B.	I	12.6	76.1	11.3	—	—	—
	II	11.0	62.5	26.4	—	—	—
A. T.	I	9.5	64.8	25.7	—	—	—
	II	8.6	74.0	17.4	—	—	—
W. D.	I	11.8	59.3	28.9	8.8	60.6	30.6
	II	8.4	61.2	30.4	6.7	72.6	20.7
D. W.	I	9.9	64.5	25.5	6.1	73.5	20.5
	II	6.5	75.8	17.7	4.8	74.9	20.3
W. V.	I	—	—	—	6.4	74.6	19.0
	II	—	—	—	5.2	74.8	20.4
R. F.	I	—	—	—	7.8	73.1	19.0
	II	—	—	—	8.4	63.0	28.6

* Values for gallbladder bile represent the average of two to three determinations in each dietary period.

‡ Values for hepatic bile represents the average for six determinations during the steady-state period of formula infusion.

and phospholipids. In five of six patients, pool sizes of bile acids were numerically greater on polyunsaturated fat, but the differences for the two groups were not significant by paired analysis. The most striking increase in pool size was noted in patient W. D. who also had a marked increment in fecal excretion of bile acids in period II.

Although polyunsaturated fats did not cause a consistent increase in the relative content of cholesterol in gallbladder bile, four patients did have a greater lithogenicity in period II. While increases in the molar percent of cholesterol might simply reflect random variation, consideration should be given to the possibility that a subgroup of patients are prone to development of abnormal bile on polyunsaturated fats. This possibility seems more likely in view of the observation that these fats may enhance formation of cholesterol gallstones (58).

One mechanism for increasing bile lithogenicity is through reduction of the pool size of bile acids (43, 44), but the present data show that this change does not occur during feeding of polyunsaturated fats. If anything, pools of bile acids tended to increase in period II, and hepatic secretion rates of bile acids and phospholipids were not decreased. Therefore, a reduction in solubilizing lipids could not account for any greater lithogenicity on polyunsaturated fats. Also, there was no selective reduction in chenodeoxycholic acid, as shown in Table VI; the percentage of chenodeoxycholic acid relative to the other bile acids in gallbladder (or hepatic bile) was not appreciably changed during period II.

Another mechanism for enhancing lithogenicity is by an increase in hepatic secretion of cholesterol (34, 59). In fact, two patients (W. D. and R. F.) had greater outputs of biliary cholesterol in period II during the infusion of formula. Also, hepatic secretion of cholesterol could have been enhanced during the fasting period; in two patients (E. McD. and J. S.), an increase in molar percentage of cholesterol was found in gallbladder bile after an overnight fast even though a comparable change did not occur in hepatic bile obtained during formula infusion. Finally, an in-

TABLE V

Effects of Fat Exchange on Hourly Outputs of Biliary Lipids and Pool Sizes of Bile Acids

Patient	Period	Biliary lipid outputs			Bile acid pool size
		Cholesterol	Bile acids	Phospholipids	
		*mg/h SD**			*mg*
W. D.	I	54±2	507±252	375±144	1,356
	II	79±10	1,115±108	496±43	2,286
	Δ‡	25§	608§	121	930
W. V.	I	52±6	784±69	309±29	1,686
	II	56±4	997±449	418±223	1,866
	Δ	4	213	109	180
J. S.	I	58±15	568±138	533±158	1,704
	II	59±12	671±269	413±228	2,107
	Δ	1	103	120	403
E. McD.	I	75±10	1,456±230	569±137	2,817
	II§	—‖	—‖	—‖	3,161
					344
D. W.	I	67±10	1,170±108	444±84	2,036
	II	61±15	1,104±94	482±55	2,091
	Δ	−6	−66	38	55
R. F.	I	109±11	1,329±275	529±55	2,577
	II	182±37	1,612±391	1,130±350	2,505
	Δ	73§	283	601§	−72

* In all patients, hourly outputs were measured during a period of 6 h formula infusion (steady state) after an initial infusion period of 4 h (equilibration period). In each case, six hourly determinations were carried out.

‡ Difference between periods I and II. A positive value indicates an increase in period II.

§ Period II significantly different from period I (P 0.05 or less) (Student's t test).

‖ Measurements of biliary lipid outputs were not completed because of technical difficulties.

crease in excretion of fecal neutral steroids found on polyunsaturated fats is compatible with an increase in hepatic secretion of cholesterol at some phase of the diurnal cycle. Thus, if polyunsaturated fats do enhance lithogenicity of bile in some patients, this increase seems more likely to be due to a greater output of biliary cholesterol than to a reduction of solubilizing lipids (bile acids and phospholipids).

Mechanism for increased fecal steroids. An increase in fecal excretion of neutral and acidic steroids on polyunsaturated fats could result from either an increment in biliary outputs of cholesterol and bile acids or a decrease in their reabsorption. In the case of cholesterol, two patients (W. D. and R. F.) had significant increases in outputs of biliary cholesterol in period II; it is of interest that these were the two patients with type V patterns. In these particular patients, it seems unlikely that the primary mechanism for an increase in fecal neutral steroids was a decrease in absorption of cholesterol, but reduced absorption cannot be entirely excluded as a contributing factor for any of the patients. Similarly, the data favors an increase in biliary outputs of bile acids as an explanation for the greater fecal excretion of acidic steroids in period II; this is best exemplified again by patient W. D. The pool size and hepatic secretion rates of bile acids in this patient were both increased markedly during polyunsaturated fat feeding. In addition, the other patients did not have decreases in either pool sizes or secretion rates of bile acids during period II, as might have occurred if reabsorption was interrupted.

DISCUSSION

Mechanism of plasma cholesterol lowering. A reduction in plasma cholesterol by polyunsaturated fats could be due to alterations in cholesterol metabolism or to changes in the metabolism of other lipoprotein constituents. The possibility that these fats might lower plasma cholesterol by affecting cholesterol metabolism itself was first examined in 1957 by Hellman, Rosenfeld, Insull, and Ahrens (3); they reported that a single patient fed polyunsaturated fats had a significantly increased excretion of neutral but not acidic steroids when plasma cholesterol levels were falling. Since the quantity of excess cholesterol that appeared in feces corresponded to the amount of cholesterol lost from the plasma, these authors suggested that the decrease in plasma cholesterol might be specifically linked to an excretion mechanism. Following this report, additional studies have claimed that ingestion of vegetable fats caused an enhanced excretion of fecal steroids (4–12, 15, 16); a rather consistent finding has been that normolipidemic subjects show a small but significant increase in fecal steroids on polyunsaturated fats (4, 10, 12, 16).

TABLE VI

Effects of Fat Exchange on Composition of Individual Bile Acids

Patient	Period	Cholic acid	Chenodeoxycholic acid	Deoxycholic acid	Lithocholic acid
		molar %			
M. P.*	I	45	23	29	3
	II	62	22	16	0
J. S.*	I	24	32	41	3
	II	61	31	7	1
J. E.*	I	31	45	22	2
	II	61	31	7	1
E. McD.*	I	31	33	35	1
	II	43	24	32	1
N. B.*	I	59	17	23	1
	II	53	30	17	0
A. T.*	I	39	23	36	2
	II	31	24	44	2
W. D.*	I	15	27	55	3
	II	51	34	15	0
D. W.*	I	38	45	15	2
	II	37	46	16	1
W. V.‡	I	26	31	43	0
	II	37	22	41	0
R. F.‡	I	19	40	39	2
	II	23	34	39	4

* Values shown for these patients are the average for two to three determinations on gallbladder bile during periods I and II.

‡ Values on patients W. V. and R. F. represent the average composition of bile acids during the steady-state period of measurement of biliary lipid outputs. These patients had previous cholecystectomy.

Despite these findings, the hypothesis that lowering of plasma cholesterol by polyunsaturated fats can best be explained by a greater excretion of steroids has been brought into question by other studies. In 1965, Spritz, Ahrens, and Grundy (13) reported that exchange of polyunsaturated for saturated fats did not cause an increase in fecal steroids in five patients. About this same time, Avigan and Steinberg (18) found no significant changes in fecal steroid excretion in five of six patients during a similar fat exchange. The patients studied in these two reports differed considerably from one another in concentrations of plasma lipids, and their lipoprotein patterns were not well defined. However, in a more recent study that included eight patients with familial hypercholesterolemia,

Grundy and Ahrens (14) again found no consistent increase in steroid excretion after institution of polyunsaturated fats. In this study, some patients showed slight increases of acidic steroids, but even when changes were statistically significant, they were of a small degree and generally could not account for the decrement in plasma cholesterol. Since most patients showed no changes, it was concluded that feeding of polyunsaturated fat need not produce an increased excretion of steroids to achieve a reduction in plasma cholesterol. The interpretation was made that the primary action of polyunsaturated fats in cholesterol lowering is not to cause an increased output of fecal steroids but to redistribute cholesterol between plasma and tissue compartments.

Since a reduction of plasma cholesterol in patients with hypercholesterolemia can occur without an enhanced steroid excretion, the question arises as to whether the increased excretions noted in the present study were casually related to cholesterol lowering. A lack of change in cholesterol balance in some patients indicates that greater outputs of fecal steroids may not be the only factor responsible for plasma cholesterol reduction; nevertheless, consideration should be given to the possibility that excess steroid excretion in hypertriglyceridemic patients could at least contribute to the decrease of plasma cholesterol. Polyunsaturated fats might influence plasma cholesterol levels through multiple mechanisms, and a greater excretion of steroids could facilitate cholesterol lowering even if it is not the only mechanism. By the same token, continuous removal of cholesterol from the body in increased amounts, regardless of its origin, reduces the availability of cholesterol for transfer to the plasma compartment. Whereas increased excretion might not be the primary event leading to the decrement in plasma cholesterol, it may be contributory by allowing this lowering to occur to a maximum extent.

If polyunsaturated fats do in fact have multiple actions, they could also cause changes in the metabolism of constituents of lipoproteins other than cholesterol. Indeed, the present study supports this possibility. The results suggest that a decrease in plasma cholesterol may be linked to alterations in triglyceride metabolism. The data show clearly that polyunsaturated fats, as compared with saturated fats, can cause a lowering of plasma triglycerides and hence very low density lipoproteins (VLDL). Although triglyceride lowering was not seen in all patients, the degree of cholesterol reduction was generally related to changes in triglyceride levels. Thus, an alteration in the metabolism of VLDL is apparently another mechanism by which levels of cholesterol in the plasma can be reduced.

Possible adverse effects of polyunsaturated fats. Regardless of mechanisms of action, polyunsaturated fats cause a significant lowering of plasma cholesterol in almost all subjects. For this reason, the use of these fats in the diet must be considered as a potentially valuable approach for decreasing one of the risk factors for atherosclerosis. However, their value should be evaluated in the light of possible adverse effects. One possible effect may be related to the fate of cholesterol lost from the plasma. If cholesterol that disappears from the plasma compartment enters directly into tissue pools, the increase in tissue cholesterol might accelerate rather than retard atherogenesis. The finding that patients with hypercholesterolemia do not excrete a quantity of cholesterol equivalent to that lost from the plasma first raised this possibility. However, the current results are not in accord with this hypothesis as a general phenomenon. In the present study on hypertriglyceridemic patients and in previous work on normal subjects (4, 10, 12, 16), plasma cholesterol reduction was usually associated with an increased steroid excretion, and this finding would favor the concept that polyunsaturated fats lower cholesterol by a relatively safe mechanism at least in these types of subjects. If the increase in fecal steroids is due to a primary mobilization of cholesterol from the body (e.g., increased biliary secretion of cholesterol or increased conversion of cholesterol into bile acids), body pools of cholesterol should be reduced. In such a case, a decrease in total body cholesterol might occur despite a compensatory increase in cholesterol synthesis. On the other hand, a more remote possibility should also be considered; if polyunsaturated fats were to cause a primary increase in cholesterol production, total body pools might be increased in spite of an increased excretion. Therefore, to fully assess the effects of these fats on cholesterol metabolism, measurements of total body cholesterol must be carried out. The findings that polyunsaturated fats cause an increased excretion of fecal steroids, decreased plasma cholesterol, and even disappearance of xanthomatosis (60) in hypertriglyceridemic patients strongly suggests that tissue pools of cholesterol are reduced; however, only direct measurements of body pools can provide proof.

Another possible side effect of polyunsaturated fats is the formation of cholesterol gallstones, as found in man by Sturdevant, Pearce, and Dayton (58) and in squirrel monkeys by Melchior, Clarkson, Bullock, and Lofland (61). In the present study, the question was examined as to whether polyunsaturated fats enhance lithogenicity of bile. The results suggest that lithogenicity of fasting gallbladder bile is increased in some patients but not all. When an increase in lithogenicity does occur, it is not the result of a reduction in pool size of bile acids; a decrease in the size of the circu-

lating bile acid pool was not found in any of our patients. Similar results have been reported by Lindstedt, Avigan, Goodman, Sjövall, and Steinberg (19) and by Hellström and Lindstedt (20); these workers found no decrease in the pool size of cholic acid after introduction of polyunsaturated fats to the diets of normal subjects and hyperlipidemic patients.

Since pool sizes of bile acids are not decreased by polyunsaturated fats, a more plausible mechanism for occurrence of lithogenic bile may be that biliary cholesterol is increased. In fact, two patients (W. D. and R. F.) had measurably greater rates of hepatic secretion of cholesterol on polyunsaturated fats. Also, a greater output of biliary cholesterol may have occurred at some time during a 24-h period even in those patients in whom it was not readily detected by secretion studies. This possibility is suggested by the observation that a greater lithogenicity on polyunsaturated fats was frequently found in gallbladder bile but not in hepatic bile that was secreted during feeding. If increased biliary cholesterol is the primary mechanism by which bile becomes more lithogenic on polyunsaturated fats, these fats should be especially likely to produce gallstones in patients who already have greater cholesterol outputs. One example could be the obese patient; we have reported that obesity increases lithogenicity of bile by enhancing hepatic secretion of cholesterol (34, 59, 62), and if cholesterol output is accentuated even more by polyunsaturated fats, gallstones could develop. Indeed, the importance of obesity as a factor for increasing gallstone formation in patients fed these fats has been shown by Sturdevant et al. (58). In their autopsy study on patients given polyunsaturated fats for long periods, the prevalence of gallstones in those who were obese was extremely high (58% of patients). Since weight reduction decreases biliary cholesterol and bile lithogenicity (62), it would seem advisable to reduce total body weight of hypertriglyceridemic patients before institution of polyunsaturated fats. As recently demonstrated by Olefsky, Reaven, and Farquhar (63), weight reduction itself causes a significant decrease in plasma lipids in patients with hyperlipidemia. After weight reduction, feeding of polyunsaturated fats should produce a further reduction in plasma lipids while the risk of gallstone formation may be lessened.

Differences between patients with hypercholesterolemia and hypertriglyceridemia. The observation that many patients with hypertriglyceridemia have an increased excretion of steroids on polyunsaturated fats whereas those with familial hypercholesterolemia generally do not suggests that metabolism of cholesterol as well as that of lipoproteins may be different in the two categories of patients. It seems unlikely that this discrepancy can be explained by the fact that formula diets were used in our previous study while a mixed diet of formula and solid food was employed in the present investigation. We previously observed that two hypertriglyceridemic patients who received pure formula diets had an increased excretion of both neutral and acidic steroids on exchange of polyunsaturated for saturated fats (14, 15), but it was not realized at the time that such a change would represent a more generalized phenomenon for this category of patient.

Several workers have reported that sterol metabolism in hypercholesterolemic and hypertriglyceridemic patients may be different in other ways; it has been claimed that both cholesterol and bile acid synthesis are increased in patients with hypertriglyceridemia, as compared to those with hypercholesterolemia (64–67). However, our cholesterol balance results do not support the claim that the majority of patients with hypertriglyceridemia have increased sterol synthesis. Table VII presents a comparison of rates of turnover of cholesterol and bile acids in patients of the present study as well as those of our previous study involving fat exchange (14). Values are presented only for the period of saturated fat feeding. The results show that turnover of both cholesterol and bile acids were roughly the same for patients with familial hypercholesterolemia and for those with hypertriglyceridemia who had type IV patterns. Clearly, most of the patients with hypertriglyceridemia did not have an excessive turnover of either cholesterol or bile acids. However, in three patients with type V patterns both cholesterol and bile acids were markedly increased. On the basis of our previous studies, it is doubtful that all patients with type V patterns have an unusually high production of sterols (68), but a subgroup of hypertriglyceridemic patients may well exist who show an increased synthesis of both cholesterol and bile acids. This tendency for overproduction in some patients with elevated triglycerides is not necessarily correlated with responsiveness to polyunsaturated fats; even some of those who did not have elevated turnover rates on saturated fats demonstrated an increased excretion of steroids on fat exchange.

In patients with hypertriglyceridemia, plasma cholesterol lowering by polyunsaturated fats seems to be intimately related to changes in triglycerides. When levels of triglycerides in these patients are not significantly reduced, little change occurs in plasma cholesterol. Polyunsaturated fats may thus reduce cholesterol concentrations in hypertriglyceridemic patients by affecting metabolism of triglycerides (or VLDL). Rates of either secretion or removal of VLDL could be affected; this possibility has been recognized pre-

TABLE VII

*Turnover of Cholesterol and Bile Acids in Patients with Familial Hypercholesterolemia and Hypertriglyceridemia**

Patient	Cholesterol turnover‡	Bile acid turnover§
	mg/kg/day	
I. Familial hypercholesterolemia|| (type II)		
1	7.4	1.6
2	14.5	5.5
3	10.0	1.0
4	9.1	2.3
5	13.8	3.9
6	12.2	2.7
7	17.7	4.7
9	12.6	3.1
Avg	12.2	3.1
II. Hypertriglyceridemia (type IV)		
E. McD.	10.4	3.4
A. T.	12.5	4.9
D. W.	13.1	3.2
W. V.	10.4	3.4
J. S.	8.5	2.3
J. E.	14.3	6.7
11	18.4	5.1
Avg	12.5	4.2
III. Hypertriglyceridemia (type V)		
10	26.1	11.5
R. F.	25.8	10.2
W. D.	17.0	9.1
Avg	23.0	10.3

* Patients represented by numerals are those from our previous publication (14). All results are those obtained during feeding of saturated fats for both studies.

‡ Cholesterol turnover is equal to the sum of endogenous neutral steroids and acidic steroids. As described previously (56), cholesterol turnover should approximate cholesterol synthesis on a cholesterol-free diet.

§ Bile acid turnover is assumed to equal the average daily excretion of bile acids in the steady state (36).

|| Patients were designated as having familial hypercholesterolemia on the basis of either or both of two criteria: (*a*) hypercholesterolemia (type II) in a primary relative, or (*b*) extensive tendon xanthomatosis.

viously by other workers (69, 70). In contrast, cholesterol reduction in normolipidemic subjects or those with familial hypercholesterolemia occurs predominately in the low density lipoprotein (LDL) fraction (71); in both groups, decreases of considerable magnitude can occur without changes in triglyceride concentrations. Although mechanisms for LDL reduction have not been fully determined, a possibility has been suggested by Spritz and Mishkel (71). They propose that unsaturated fats cause an alteration in configuration of lipids (cholesterol esters and phospholipids) within LDL so that the capacity for cholesterol transport is reduced.

In review of the results of the present and previous studies, it is apparent that the response to polyunsaturated fats varies from patient to patient. Some patients, particularly those with familial hypercholesterolemia, usually have little or no change in cholesterol balance when polyunsaturated fats are exchanged for saturated fats. Other patients, especially those with hypertriglyceridemia, have a distinct increase in fecal steroids on polyunsaturated fats. Another group, including many normal subjects, show significant but quantitatively small increments in either neutral or acidic steroids. Whereas increased outputs of steroids may contribute to the hypocholesterolemic action of polyunsaturated fats, there is not a close correlation between the decrement of plasma cholesterol and the increment in fecal steroids. Indeed, an increased excretion is not an absolute requirement for plasma cholesterol reduction.

This variability of response also suggests that polyunsaturated fats may produce multiple metabolic changes, as compared with saturated fats. When polyunsaturated fats are ingested, the fatty acid composition of many lipids (cholesterol esters, triglycerides, and phospholipids) are changed, and physical properties of tissue and plasma lipids are consequently altered. It would therefore not be surprising if changes occurred in rates of metabolic processes involving these lipids. For this reason, it may be futile to search for a single or unifying explanation for the lowering of plasma cholesterol by polyunsaturated fats. The magnitude of the decrease in plasma cholesterol may be the result of changes in the metabolism of cholesterol, bile acids, and glycerides, as well as being dependent on the way in which lipids are related sterically to one another in lipoproteins. Likewise, the response to polyunsaturated fats appears to vary between different types of subjects, and the predominate mechanism affecting plasma cholesterol concentrations may also depend on the presence or absence of particular metabolic defects.

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