Increased Sterol Excretion with Polyunsaturated-Fat High-Cholesterol Diets

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Previous studies have shown that polyunsaturated ruminant fats in the diets of human subjects cause an increase in cholesterol and bile acid excretion during the first 3 weeks of such diets. The present studies were designed to compare the effects of polyunsaturated (P) and conventional (S) ruminant fats at two levels of dietary cholesterol intake: a higher (HC) and lower (LC). Four study periods, each of about 3 weeks' duration, were conducted in 5 healthy subjects providing these dietary combinations: HCS, HCP, LCS, LCP. Neutral sterols and bile acids were measured in the feces, and sterol balances were calculated.

Plasma cholesterol levels were significantly lower with P than with S diets at both HC and LC intakes. Changes attributable to differences in fatty acids and to differences in cholesterol intake appeared to exert independent effects. The major changes occurred in lipoproteins with density 1.019–1.045.

Cholesterol absorption expressed as a percentage of the dietary intake was not significantly different with the four diets. Neutral sterol excretion of probable endogenous origin and bile acid excretion were significantly higher during the HCP than during the HCS periods, but the difference between LCP and LCS periods was less marked. Net sterol excretion was therefore significantly greater with HCP and LCP than with HCS and LCS diets, the differences being greater at HC than at LC intakes. Comparisons of diets with similar fatty acid but differing cholesterol intakes showed lower net sterol excretion with HCS than with LCS diets (presumably due to suppression by HC of cholesterol synthesis), but this difference was not seen between HCP and LCP diets. This finding, together with greater sterol excretion with HCP than with HCS diets, showed that enhanced sterol excretion with polyunsaturated fat was potentiated with higher cholesterol intake. This enhanced excretion was generally greater during the first than during the second 3-week period of polyunsaturated fat.

THE SUBSTITUTION of polyunsaturated fat for saturated fat in the diet lowers plasma lipids through mechanisms that are not well defined. Several recent studies, using reliable sterol balance techniques, have reported enhancement of sterol excretion.[1–3] Although Grundy and Ahrens[4] have discounted this as an adequate explanation for the cholesterol-lowering effect of polyunsaturated fats, almost half of their patients did show an increase in bile acid excretion. The somewhat lower proportion of subjects showing this response in their study, as compared to other studies, may reflect their inclusion of many subjects with familial hypercholesterolemia, who may excrete low amounts of bile acids for genetic reasons.[5–7]

We have shown in two previous studies[3,8] utilizing polyunsaturated or saturated ruminant fats[9] that sterol excretion in normal healthy subjects was increased with polyunsaturated fat during the first 3 weeks while the plasma cho-

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lesterol level was falling,[3] but that in the new steady state, sterol excretion was similar with polyunsaturated and saturated fats.[8]

We have explored this question further, extending it to include a study of the possible interaction of dietary cholesterol with the degree of unsaturation of the fat. This has previously not been specifically examined in relation to sterol excretion, although polyunsaturated fat has been reported to stimulate bile acid and endogenous cholesterol excretion both in the absence[2] and in the presence of dietary cholesterol.[1,3,4]

The purpose of the present study was threefold: (1) to examine the relative effect of dietary cholesterol and the fatty acid composition of ruminant fats on the plasma cholesterol level; (2) to reexamine the enhancement of sterol excretion with polyunsaturated fats in relation to the duration of the diets; (3) to study the interaction of cholesterol and fat in the diet with respect to sterol excretion.

METHODS

Diets

Five healthy men aged 17 to 22 years were studied on a metabolic ward. Four were within 10% of their standard weight,[10] and one was 21 kg overweight (Table 1). Their daily caloric requirements were estimated beforehand, and a constant weight was subsequently maintained by minor adjustments in the carbohydrate moiety of the diet.

There were four dietary periods, which were designed to compare polyunsaturated with more saturated fatty acids at two intakes of cholesterol. All fats were of animal origin and consisted of conventional or polyunsaturated dairy fat, beef, and lamb. These products have been described in our previous papers;[3,8,9] apart from their potential use in lipid-lowering diets, they allow comparisons to be made of fats that differ only in their fatty acid composition. The major differences between the polyunsaturated and saturated fats were in the proportions of linoleic acid (8.0% vs. 1.2% of total calories), palmitic acid (6.4% vs. 10.5%), and myristic acid (1.6% vs. 2.7%). The respective ratios of linoleic acid to the two saturated fatty acids were 1.00 and 0.09. The higher and lower intakes of cholesterol were within the range commonly eaten in the community and differed by about 250–300 mg/day (Table 3).

Equations have been derived by others for changes in the plasma cholesterol concentration that might be brought about by differences in the fatty acid composition[11] and the cholesterol content[12] of foods. The predicted differences in our studies were 20 mg/100 ml attributable to the unsaturation of the fat and 10.6 mg/100 ml due to the cholesterol intake.

Table 1. Plasma Cholesterol and Triglyceride Concentrations during Four Dietary Periods Differing in Cholesterol Content and Fatty Acid Composition

Subject	Weight (kg)	Plasma Cholesterol Concentration (mg/100 ml)				Plasma Triglyceride Concentration (mg/100 ml)			
		HCP*	HCS*	LCP*	LCS*	HCP	HCS	LCP	LCS
RN	95	169	177	162	177†	298	228	231	473†
JP	65	160	204	158	175	81	83	76	81
BC	75	196	210	179	201	88	82	84	113
GR	75	183	198	178	227	114	123	99	115
NV	63	188	198	172	181	103	98	102	85
Mean		177	197	169	192	136	123	118	173
±SD		14	12	8	22	91	61	64	168

The four dietary periods were randomized and not eaten in the order shown here.

*HC = higher cholesterol; LC = lower cholesterol; P = polyunsaturated fat; S = saturated fat.

†Because of the higher triglyceride value in RN in the LCS period, the LDL cholesterol concentration was used in the HCS-LCS paired t-test comparison in the subject.

The fat-containing foods included milk, cream, cheese, ice cream, beef, lamb, and beef drippings. This provided 42%–44% of the total calories as fat and the entire intake of cholesterol during the periods of lower intake. The higher cholesterol intake was provided by the addition of dried egg yolk. The absolute amounts of cholesterol were proportional to total calories and therefore to body weight. Fifteen percent of calories were derived from protein and the remaining 41%–43% from carbohydrate. (The proportions derived from the various sugars and from starch were comparable in the four diets.)

The first of the four diets was the higher cholesterol saturated-fat (HCS) diet in all subjects, because it resembled their previous food intake. It lasted 19 days and was followed by the other three periods each of 23 days. The lower cholesterol polyunsaturated-fat diet (LCP) was eaten during the third period by all subjects; this ensured that polyunsaturated fats would be eaten for 46 consecutive days. The remaining diets, the lower cholesterol saturated-fat (LCS) diet and the higher cholesterol polyunsaturated-fat (HC) diet, were assigned randomly as shown in Table 3.

Laboratory Procedures

Blood was obtained three times weekly immediately after the subjects woke in the morning after a 12–14-hr fast. Plasma lipoproteins were separated twice during the latter part of each dietary period; the following lipoprotein classes were harvested by the technique of Havel, Bragdon, and Boyle:[13] $d <$ 1.006 (VLDL); $d = 1.006$–1.019 (LDL1); $d = 1.019$–1.045 (LDL2); $d = 1.045$–1.063 (LDL3); $d > 1.063$ (HDL). The recovery of lipoproteins, as estimated from the sum of the cholesterol content in each class, was 85%. Cholesterol and triglyceride concentrations were measured in whole plasma and in lipoproteins in a Technicon II autoanalyzer.

Feces were collected during the last 8 days of each dietary period and pooled into 2-day aliquots. The homogenized feces were treated as described by Grundy, Miettinen, and Ahrens[14,15] to quantify the daily excretion of neutral sterols and bile acids. Recoveries were checked by including radioactive cholesterol and cholic acid through the procedure, and they averaged 92% and 85%, respectively. The final gas-chromatographic analysis was carried out on the trimethylsilyl derivatives of cholesterol, coprostanol, and coprostanone (and the corresponding plant sterols) and of the total bile acids; 5α-cholestane was used as internal standard. The daily excretion rate was derived from the recovery of chromic oxide,[16] 400 mg of which was taken daily as a marker of fecal flow. Recoveries of chromium oxide for the 5 subjects averaged 393, 398, 419, 434, and 375 mg/day. Intestinal degradation and loss of neutral sterols[17] had not occurred in the two previous studies with these foods. In the present study, 1 μCi ^{14}C-4-β-sitosterol was given orally at the beginning of each period of fecal collection together with 4 μCi ^{3}H-1,2-cholesterol for the measurement of cholesterol absorption.[18] (Both isotopes had been obtained from Radiochemicals, Amersham, England, and were purified by silica-gel chromatography.) Recovery of β-sitosterol for all subjects averaged 87% (range 79%–96%). The average daily intake of β-sitosterol was 75 mg.

RESULTS

Plasma Lipids and Lipoproteins

Table 1 shows the mean plasma cholesterol and triglyceride levels measured on five occasions during the latter 10 days of each study. The mean values for cholesterol for the group showed higher concentrations with saturated than with polyunsaturated diets: the average differences of 20 and 23 mg/100 ml for the comparisons at the higher and lower cholesterol intakes, respectively, were both significant ($p < 0.05$ by paired t-test analysis). The observed differences due to the change in the dietary fatty acid composition were in close agreement with the expected differences based on the Keys, Anderson, Grande[11] equation (20 mg/100 ml at both intakes of cholesterol). When the differences due to fatty acid composition were pooled (10 comparisons, 5 at higher and 5 at lower cholesterol intakes) the level of significance was $p < 0.005$.

The fasting plasma triglyceride levels were not significantly altered by the changes in dietary fatty acid composition; statistical analysis in this small group

of subjects was complicated by the inclusion of one overweight, hypertriglyceridemic subject.

Differences due to different intakes of cholesterol were analyzed and compared with expected differences based on the equation of Mattson, Erickson, and Kligman.[12] The very high triglyceride value in subject RN during the lower cholesterol saturated-fat diet distorted the comparison for the saturated-fat diets; since this led to a higher concentration of cholesterol in VLDL, we have used the values in LDL for this individual. The higher cholesterol intakes led to higher mean cholesterol levels with both the saturated- and polyunsaturated-fat diets. The mean differences between the higher and lower cholesterol intakes of 8.6 and 8.8 mg/100 ml with the polyunsaturated- and saturated-fat diets, respectively, reached the 5% level of significance only with the polyunsaturated-fat diet. Of the 10 comparisons, there were 7 occasions when periods of higher and lower intakes of cholesterol (but with similar fatty acid composition) occurred consecutively; the mean observed difference in the plasma cholesterol level due to the change in cholesterol intake was 12.3 mg/100 ml in these 7 comparisons, which was significantly different at the 2% level and was in close agreement with the calculated difference of 10.6 mg/100 ml.

These studies have therefore confirmed our previous findings of the cholesterol-lowering potential of polyunsaturated ruminant fats, which resembles the effect obtained with a similar change in polyunsaturation based on vegetable oils. The influence on the plasma cholesterol level attributable to dietary cholesterol was not as clear cut; the reasons for this probably include the small number of subjects, the relatively small difference in cholesterol intake, and variability in subject response. Nevertheless, the 7 comparisons that allowed observations to be made in consecutive dietary periods showed significantly higher plasma cholesterol levels with higher cholesterol intakes, showing that the cholesterol content and the fatty acid composition of fats exerted independent effects on the plasma cholesterol concentration.

The cholesterol and triglyceride concentrations in the plasma lipoproteins were measured twice during the last week of each dietary period in every subject. The results for the whole group (Table 2) show that the higher plasma cholesterol concentration that occurred with dietary saturated fats was attributable largely to changes within low-density lipoproteins ($p < 0.02$, on paired t-test analysis).* Within the class of lipoproteins, the mean change was greatest in lipoproteins within the density range of 1.019–1.045 (termed LDL2).† There was virtually no change for the group as a whole in the cholesterol concentrations in very low density and high-density lipoproteins. Nevertheless, the small individual changes in high-density lipoproteins might be relevant: the correlation between the percentage cholesterol changes in whole plasma and in low-density/high-density

*The mean difference between the polyunsaturated- and saturated-fat diets was 13 mg/100 ml for the lipoproteins and 20 mg/100 ml for whole plasma. This reflects the 85% recovery of cholesterol in the separated lipoproteins and the smaller number of observations on lipoproteins versus whole plasma.

†There was a significant inverse correlation between the cholesterol concentrations in LDL2 and LDL3 ($r = -0.64$); it is uncertain whether this reflects technical or biological factors.

Table 2. Changes Due to Dietary Fatty Acid Composition in the Cholesterol Concentration in Plasma Lipoproteins

Subject	Dietary Comparison	Difference in Cholesterol Concentration (mg/100 ml) VLDL*	LDL1*	LDL2*	LDL3*	Total LDL*	HDL*
RN	HCS-HCP	-21	+4	- 2	+ 4	+ 6	+4
	LCS-LCP	+16	- 1	+ 2	- 14	- 13	- 2
JP	HCS-HCP	+ 4	+5	+ 1	+20	+26	+1
	LCS-LCP	- 6	+1	+32	- 8	+25	+1
BC	HCS-HCP	0	+3	- 15	+18	+ 6	0
	LCS-LCP	+ 2	- 1	+28	- 2	+25	+1
GR	HCS-HCP	+ 3	+1	- 21	+26	+ 7	- 2
	LCS-LCP	+ 1	+1	+31	- 1	+31	+5
NV	HCS-HCP	- 4	0	+23	- 4	+19	- 5
	LCS-LCP	- 2	+1	+ 7	- 5	+ 3	+2
Mean		- 0.7	+1.4	+ 8.7	+ 3.4	+13.5†	+0.5

*Lipoprotein densities: VLDL < 1.006; LDL1 = 1.006–1.019; LDL2 = 1.019–1.045; LDL3 = 1.045–1.063; HDL > 1.063.

†Significantly higher with saturated- than with polyunsaturated-fat diets ($p < 0.02$).

lipoproteins was much higher ($r = +0.864$; $p < 0.01$) than that between the percentage changes in whole plasma and low-density lipoprotein alone ($r = +0.353$).

The changes in cholesterol levels attributable to differences in cholesterol intake were also largely confined to the low-density lipoproteins, with density of 1.019–1.045.

Sterol Metabolism

The complete data are presented in Table 3, and the differences due to dietary change are shown in Table 4. Cholesterol absorption varied from 30% to 61% and was not significantly different with polyunsaturated and saturated fats. With both kinds of fats the fractional absorption was a little higher at the lower than at the higher cholesterol intake, although not significantly so. It should be noted, however, that the percentage absorption tended to be higher with the saturated fats.

Bile acid excretion was higher with polyunsaturated than with saturated fats in all subjects during the periods of higher cholesterol intake, the mean values being respectively 381 and 281 mg/day ($p < 0.01$). At the lower cholesterol intake, bile acid excretion was not significantly different with the two kinds of fat. Different intakes of cholesterol did not significantly influence bile acid excretion, although during the periods of polyunsaturated fat, bile acid output was greater at the higher intake of cholesterol in 4 of the 5 subjects, though the overall difference was not significant.

Neutral sterol excretion, which includes unabsorbed dietary cholesterol and endogenous cholesterol, was significantly higher during the two polyunsaturated-fat periods than during the two saturated-fat periods ($p < 0.01$). Since the amounts of cholesterol eaten and the proportions absorbed with the corresponding polyunsaturated- and saturated-fat diets were not significantly

Table 3. Sterol Metabolism during the Last 8 Days of Each 23-Day Dietary Period

Subject	Dietary Period (order)*	Cholesterol Intake (mg/day)	Cholesterol Absorption (%)	Cholesterol Absorption (mg)	Sterol Excretion (mg/day†) Neutral	Bile Acid	Net
RN	HCP (4)	849	37	314	1428 ± 267	291 ± 62	870
	HCS (1)	836	42	351	1092 ± 88	238 ± 24	494
	LCP (3)	561	35	196	1140 ± 105	365 ± 150	944
	LCS (2)	532	49	260	924 ± 140	297 ± 56	689
JP	HCP (4)	762	58	442	569 ± 13	429 ± 39	236
	HCS (1)	754	54	407	558 ± 117	352 ± 70	156
	LCP (3)	474	64	303	453 ± 121	340 ± 97	319
	LCS (2)	458	56	256	470 ± 193	317 ± 83	329
BC	HCP (2)	804	34	273	827 ± 87	338 ± 22	361
	HCS (1)	787	42	330	706 ± 59	220 ± 47	139
	LCP (3)	552	42	232	931 ± 168	325 ± 20	704
	LCS (4)	503	56	282	627 ± 188	230 ± 27	354
GR	HCP (2)	810	30	243	1204 ± 180	636 ± 76	1030
	HCS (1)	812	42	341	868 ± 68	456 ± 71	512
	LCP (3)	552	33	182	951 ± 131	403 ± 38	802
	LCS (4)	510	61	311	751 ± 80	469 ± 98	710
NV	HCP (2)	754	39	294	1165 ± 195	213 ± 50	624
	HCS (1)	754	42	316	808 ± 134	141 ± 30	195
	LCP (3)	460	49	225	762 ± 89	136 ± 28	438
	LCS (4)	464	41	190	597 ± 63	173 ± 26	306

*The order in which the various diets were tested is shown in parentheses.

† Mean ± SD of four 2-day pools.

different, we conclude that polyunsaturated fats enhance endogenous cholesterol excretion. This enhancement averaged 202 mg/day for the whole group and occurred at both intakes of dietary cholesterol (in 9 of the 10 comparisons). Thus increased bile acid excretion with polyunsaturated fat was observed in all 5 subjects only at the higher intake of cholesterol, whereas endogenous cholesterol excretion was found to be increased also at the lower intake of cholesterol.

Table 4. Mean Values (± SD) and Significant Differences for Cholesterol Absorption, Bile Acid, Neutral Sterol, and Net Sterol Excretion in the Four Dietary Periods

Dietary Period	Cholesterol Absorption	Sterol Excretion (mg/day) Neutral	Bile Acid	Net
HCP	39.6 ± 10.8	1038 ± 339	381 ± 162	624 ± 332
HCS	44.4 ± 5.4	806 ± 192	281 ± 123	299 ± 195
LCP	44.6 ± 12.5	847 ± 257	315 ± 103	642 ± 262
LCS	52.6 ± 7.8	673 ± 171	297 ± 111	477 ± 203
Significant difference†		HCP + LCP vs. HCS + LCS $p < 0.01$	HCP vs. HCS $p < 0.01$	HCP vs. HCS $p < 0.02$; LCP vs. LCS $p < 0.05$; HCP + LCP vs. HCS + LCS $p < 0.01$; LCS vs. HCS $p < 0.01$

† Paired t-test analysis.

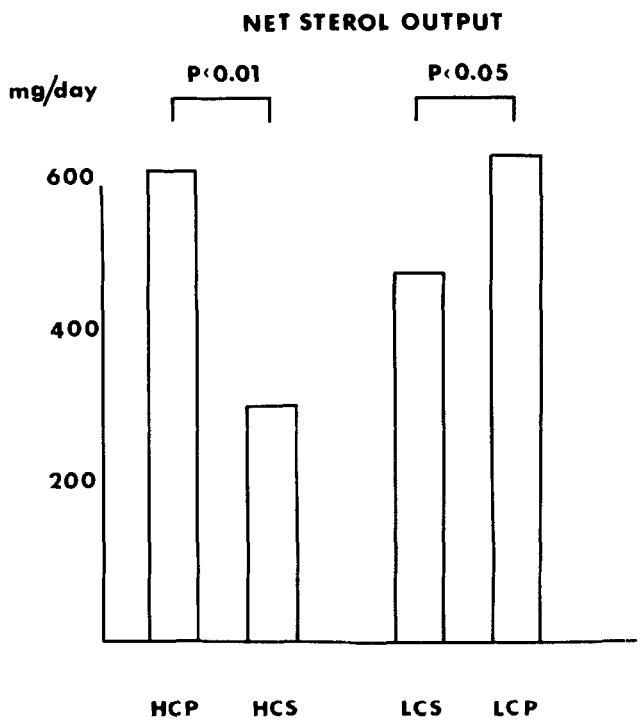

Fig. 1. Mean fecal net sterol output (mg/day) in the 5 subjects measured during final 8 days of each of four dietary periods (HCP: higher cholesterol polyunsaturated fat; HCS: higher cholesterol saturated fat; LCS: lower cholesterol saturated fat; LCP: lower cholesterol polyunsaturated fat).

The net sterol excretion (Fig. 1) was also significantly higher with polyunsaturated fats. This was statistically significant both at the higher and the lower intake of cholesterol ($p < 0.02$ and < 0.05, respectively). When the 10 comparisons were analyzed together, net sterol excretion was significantly higher with the polyunsaturated than with the saturated fats ($p < 0.01$). The effect of dietary cholesterol intake on net sterol excretion was different for the two fat diets. Net sterol output was significantly higher at the lower than at the higher cholesterol intake ($p < 0.01$) only with the saturated fat. This was not unexpected, since net sterol excretion is equivalent to cholesterol synthesis under steady-state conditions when a higher intake of cholesterol would lead to a partial suppression of synthesis (although it is not known whether cholesterol metabolism was in steady state during the last 8 days of any of the 23-day dietary periods). By contrast, during the periods of polyunsaturated fat, net sterol excretion was similar at both the higher and lower intakes of cholesterol, suggesting that the increase in sterol excretion attributable to the polyunsaturated fat exceeded the possible suppression of cholesterol synthesis due to the higher intake of cholesterol.

DISCUSSION

The plasma cholesterol was lowered in these studies both as the consequence of increasing the polyunsaturated-to-saturated fatty acid ratio and by decreasing the cholesterol content of the diets. Our studies confirmed that these two dietary factors exerted independent influences on the plasma cholesterol level, as demonstrated by Hegsted et al.[19] for vegetable oils containing added cholesterol. Others, such as Connor, Stone, and Hodges[20] and Erickson et al.,[21] had claimed

that changing the saturation of the fat exerted little influence on the plasma cholesterol if the diet did not also contain cholesterol. Brown[22] had also published data that suggested an interaction between dietary cholesterol and fatty acid saturation. However, a later report by Connor and co-workers[2] demonstrated a cholesterol-lowering effect with a highly polyunsaturated cholesterol-free diet.

Both dietary factors exerted their influence predominantly on the cholesterol in the low-density lipoproteins. Spritz and Mishkel[23] had previously demonstrated this with substitutions in dietary fatty acids. Although the changes in high-density lipoprotein cholesterol was not affected significantly by the saturation of the fat, the reciprocal changes between the high- and the low-density lipoproteins resulted in a high degree of correlation between the percentage changes in cholesterol in the whole plasma and in the proportions carried in low-density/high-density lipoproteins. The changes in plasma cholesterol brought about by different intakes of cholesterol were also reflected mainly in the low-density lipoproteins. Both kinds of dietary change affected mainly the lipoproteins in the density range 1.019–1.045.

The sterol-balance data confirmed our previous findings of increased net sterol excretion (total sterol excretion minus dietary cholesterol) with polyunsaturated ruminant fats. In both the present and previous studies there was considerable interindividual variation: the increase in sterol excretion did not necessarily include an effect on both neutral sterols and bile acids. The effect was seen in all subjects during the 3rd week of such diets,[3] but not in an additional 7 subjects studied only during the 6th week.[8] Two other studies in humans, which have shown greater excretion of sterols with polyunsaturated than with saturated fats, were also carried out for periods of 3 weeks.[1,2] Grundy and Ahrens[4] made measurements at about 3 weeks and in some subjects also several weeks later: of 11 subjects, 5 showed increased bile acid excretion, and 3 showed increased total endogenous sterol excretion. Recent published studies in animals also suggest that the cholesterol-lowering effect of polyunsaturated fats may be partly mediated through increased excretion of sterol.[24,25]

In the present studies, polyunsaturated fats stimulated sterol excretion, as measured by three parameters. First, bile acid excretion was enhanced in all 5 comparisons made at the higher intake of cholesterol, though in only 3 of 5 at the lower intakes. Second, neutral sterol excretion was greater with polyunsaturated fat in 9 of 10 comparisons. Since the intake and the absorption of cholesterol were not significantly different during corresponding periods of polyunsaturated- and saturated-fat diets, the difference in total neutral sterol excretion would have largely reflected the output of endogenous cholesterol. Third, when considering the 10 comparisons together, net sterol excretion was also significantly higher with polyunsaturated fat (being in fact greater in 9 of 10 comparisons). Although cholesterol absorption was not statistically significantly different with the two fats, it tended to be higher with the saturated fat; and this could have contributed to the lesser excretion of neutral sterols during the saturated fat diets, especially if the reabsorption of endogenous cholesterol was also affected.

The daily net excretion of sterols averaged 254 mg (including the single comparison in which the value was higher with saturated fat: LCS-LCP in subject JP). The mean decrement in the plasma cholesterol during the 10 comparisons was

21.5 mg/100 ml, or about 600 mg assuming a plasma volume of about 3 liters. It is therefore possible for the amount that was lost from the plasma to have been cleared from the body in about 3 days. Calculations of this sort do not take into account the rate of flux of cholesterol through plasma, the possible replacement of cholesterol lost from the plasma with cholesterol from tissues, changes in the rates of turnover, synthesis and catabolism of cholesterol, etc. That does not necessarily imply that increased sterol excretion is the only mechanism or that it is the initial process through which the cholesterol-lowering effect of polyunsaturated fat is mediated.

Grundy and Ahrens[4] point out that other mechanisms must apply in those subjects in whom the fall in plasma cholesterol is not accompanied by a rise in sterol excretion, and they postulate a transfer from plasma to tissues such as the liver. This may indeed be the initial event, which is followed in normal subjects by the excretion of this sterol. In subjects with familial hypercholesterolemia (such as were studied by Grundy and Ahrens[4]) in whom the catabolism of cholesterol may be lower than normal,[5–7] the reexcretion of sterol may be diminished or delayed.

If unloading of cholesterol does indeed occur in normal subjects eating polyunsaturated fat, then our previous two studies suggest that this occurs rapidly during the first few weeks while the plasma cholesterol is falling, since the later study, carried out during the 6th week of the diets, showed similar rates of excretion with saturated and polyunsaturated fat. However, it could be argued that even after the initial rise in sterol excretion had subsided the subsequent "normal" rate of excretion is in fact still raised in relation to the reduced pool of plasma cholesterol. In the present study, two periods of polyunsaturated fat followed each other in all subjects; the diets containing higher amounts of cholesterol were eaten first three times, and those containing lower amounts twice. Thus, although polyunsaturated fat was eaten for 46 consecutive days, the plasma cholesterol was not in a steady state throughout. Nevertheless, net sterol excretion was less during the second than during the first polyunsaturated period of 4 of 5 subjects, supporting our previous observation. Despite this, the net sterol excretion during the second polyunsaturated-fat period invariably exceeded the excretion during the corresponding saturated-fat period.

The increased excretion of neutral sterols with polyunsaturated fat, taken together with the known enhancement brought about by dietary cholesterol,[26] strongly suggests an interaction between dietary cholesterol and polyunsaturated fat on neutral sterol excretion. This might have been responsible for the net sterol excretion not being less with the higher cholesterol than with the lower cholesterol intake during polyunsaturated-fat periods, since the higher intake of cholesterol might have been expected to reduce the synthesis of cholesterol, such as appeared to occur with the saturated-fat diets.

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