Sterol balance in hyperlipidemic patients after dietary exchange of carbohydrate for fat

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Abstract Dextrose was exchanged isocalorically for polyunsaturated fat in the liquid formula diets of 10 hyperlipidemic patients maintained under metabolic steady state conditions. Carbohydrate caused an increase of plasma triglycerides in all 10; plasma cholesterol rose in 7, and 6 of these 7 failed to show any increase in total fecal excretion of cholesterol. In contrast, fecal steroid excretion increased significantly in the three patients who maintained an unchanged or lower plasma cholesterol on the high-carbohydrate diet. Squalene, an obligatory precursor in the biosynthesis of cholesterol, rose in the plasma during carbohydrate feeding in 6 out of 6 patients studied.

After a single intravenous infusion of radioactive cholesterol, plasma and feces were analyzed for specific activity over at least a four-month period. Plasma and fecal neutral sterol specific activity were essentially equivalent at all times in all patients, regardless of feeding regimen. On institution of carbohydrate feeding, the slope of cholesterol specific activity flattened for those seven patients who had a rise in plasma cholesterol concentration. There was no change in slope for the two patients with fixed plasma cholesterol levels nor for the one patient with a decreased plasma cholesterol on the high-carbohydrate diet.

These experiments demonstrate a divergent response between plasma cholesterol concentration and cholesterol excretion. To establish causal relationship between the two (i.e., plasma cholesterol increases because excretion does not increase) will require further research. The flattening of specific activity die-away curves with rising cholesterol concentrations is best explained by mobilization of slowly turning-over tissue cholesterol into plasma. A subsequent decrease in cholesterol synthesis would also add to the slower decline of plasma cholesterol specific activity.

Supplementary key words Cholesterol synthesis, excretion • tissue flux • plasma specific activity decay • adipocytes • squalene • lipoproteins • uric acid • fecal beta-sitosterol recoveries • triglycerides • bile acids • olive oil

The important question whether intake of animal fats plays a causal role in the development of arteriosclerosis is widely debated (1). If lowering plasma cholesterol levels by reduction of saturated fats and cholesterol in the diet is desirable in terms of disease prevention, then another question arises, namely, whether polyunsaturated fat or carbohydrate is the more effective caloric substitute.

Isocaloric exchange of dietary carbohydrate for fat causes an increase in plasma triglycerides (2–4). The expansion in VLDL-triglyceride pool size is associated with a reduced fractional removal rate (5–7). By comparison, relatively little attention has been given to carbohydrate effects on cholesterol metabolism. Although plasma cholesterol levels may decrease when patients substitute carbohydrate for their usual dietary fat (8–10), this intervention also eliminates cholesterol from the diet. Since it is generally accepted that dietary cholesterol is one of the important determinants of plasma cholesterol concentrations (11, 12), the effect of dietary carbohydrate substitution alone on cholesterol metabolism is not clear. When sucrose was exchanged for polyunsaturated fat in diets maintaining constant cholesterol intake, both triglycerides and cholesterol increased in the plasma of four healthy volunteers (13).

The present investigation describes the effects of isocaloric exchange of carbohydrate for polyunsaturated fat in cholesterol-free liquid formula diets. Ten patients with various forms of hyperlipidemia were carefully maintained in energy balance for periods of at least 2 months on each diet. Combined isotopic and sterol balance methods provided measurements of daily cholesterol and bile acid synthesis rates and of fecal plant sterol recoveries. In addition, analyses of plasma lipoproteins, triglycerides, uric acid, and squalene were performed.

The data to be presented show that under these experimental conditions carbohydrate induction consistently elevated plasma triglycerides, while plasma cholesterol concentrations rose, remained unchanged, or fell. Concomitantly, fecal sterol excretion fell, remained unchanged or rose, respectively. Although cholesterol synthesis may increase with carbohydrate induction of triglycerides, this has not been established under the prevailing unsteady state condi-

Abbreviations: GLC, gas–liquid chromatography; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

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TABLE 1. Clinical Data

Patient	Age	Sex	Weight	Height	% of Desirable Weight[a]	Diets[b]	Diagnosis[c]
	yr		*kg*	*cm*			
1. R.C.	47	F	54	158	102	a. 70% cottonseed b. 20% cottonseed	FHC + HTG (IIb); tendinous xanthomatosis
2. J.G.	55	M	63	176	97	a. 70% cottonseed b. 20% cottonseed	HC; A.S.H.D.
3. F.G.	63	F	67	157	124	a. 70% cottonseed b. 20% cottonseed	FHC + FHTG (IIb); A.S.H.D.; cerebral arteriosclerosis; obesity
4. T.N.	39	M	85	180	114	a. 70% cottonseed b. 20% cottonseed	FHC (IIa); Tendinous xanthomatosis
5. A.G.	53	M	70	169	111	a. 45% safflower b. 45% olive c. fat-free	HC + HTG (IIb); tuberous xanthomatosis; generalized arteriosclerosis
6. D.B.	47	M	62	175	93	a. 40% corn b. fat-free	HC + HTG (III); peripheral vascular disease
7. R.B.	59	F	52	158	100	a. 45% safflower b. 45% olive c. fat-free	FHC (IIa); A.S.H.D.; rheumatic heart disease
8. R.T.	48	M	65	164	108	a. 40% corn b. fat-free	HTG (IV); A.S.H.D.
9. R.H.	42	M	89	175	133	a. 70% cottonseed b. 20% cottonseed	HC + HTG (III); tuberoeruptive and planar xanthomatosis; obesity
10. H.S.	61	M	85	169	133	a. 70% cottonseed b. 20% cottonseed	HC + HTG (IV–V); A.S.H.D.; obesity; hypertension; gout

[a] According to Metropolitan Life Insurance Co. Statistical Bulletin 40: Nov.–Dec. 1959.

[b] Eucaloric diets containing the listed percentage of total calories as fat; protein always contributes another 15% of calories; dextrose, the remainder.

[c] Abbreviations: A.S.H.D. = Atherosclerotic heart disease. Lipoprotein phenotypes of IIa, IIb, III, IV, are based on W.H.O. classification (1970, Bull. W.H.C. 43: 891). HC = hypercholesterolemia. HTG = hypertriglyceridemia. FHC, FHTG = familial hypercholesterolemia and/or hypertriglyceridemia as judged by plasma lipid concentrations at least 1 standard deviation greater than the age-adjusted mean in at least two first degree relatives. There were no proven cases of sporadic hyperlipidemia; patients not classified as familial did not have appropriate family studies performed.

tions following dietary intervention. If increased synthesis does indeed occur, then the body's capacity to excrete this additional cholesterol load may eventually determine the plasma cholesterol concentration in the new steady state.

METHOD

Patients

Ten patients were hospitalized on the metabolic ward of The Rockefeller University Hospital for periods of 4 to 6 months. Clinical data describing age, sex, body weight, diet and type of hyperlipoproteinemia are given in **Table 1**. All patients were fully ambulatory; none had congestive heart failure; no specific medications were required except for treatment of angina pectoris.

Diets

Total food intake consisted of liquid formula feedings, 5 times daily, supplemented with vitamins and minerals, as described elsewhere (14). Sodium intake was 1 g or less in all cases. Caloric intake was adjusted during the first two weeks of hospitalization in order to maintain each patient in energy balance. Body weights varied less than ± 1.5 kg thereafter.

All formulas provided 15% of total calories as milk protein (RI-5, Ross Laboratories, Columbus, Ohio); 15–85% of calories as dextrose; 0–85% as vegetable oil (corn, cottonseed, olive or safflower. See Table 1). Dietary cholesterol never exceeded 9 mg per 500 calories. Total plant sterol intake for any given patient was kept constant during all feeding periods by addition of 90% β-sitosterol, 10% campesterol–stigmasterol (kindly supplied in purified microcrystalline form by Dr. Erold R. Diller, Eli Lilly Co., Indianapolis, Indiana) to the low-fat formulas. When fat-free diets were given, plant sterols were dispensed in capsules with the usual five daily feedings. Formulas were usually prepared in 40 kg batches, and each was analyzed for consistency of sterol concentrations prior to use by previously described GLC methods (15).

Radioactive isotopes

Either [4-^{14}C]cholesterol or [1,2-^{3}H]cholesterol (New England Nuclear Corp., Boston, Mass.) was administered intravenously to each patient shortly after weight stabilization was achieved. The isotopic materials were first purified by thin-layer chromatography as described previously (15), and

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TABLE 2. Plasma chemistries and cholesterol kinetics

Patient	Diet	Plasma				Change in Plasma Cholesterol Specific Activity Slope
		Triglycerides[a]	Cholesterol[a]	Uric Acid[b]	Squalene	
		mg/100 ml	*mg/100 ml*	*mg/100 ml*	*μg/100 ml*	
1. R.C.	Fat	160 ± 26 (18)	434 ± 19	5.0	28.4 ± 4.9 (3)	
	CHO	541 ± 66 (9)[e]	307 ± 10[e]	5.5	78.2 ± 17.7 (3)[e]	Unchanged
2. J.G.	Fat	88 ± 18 (22)	194 ± 11	3.5	19.5 ± 5.2 (5)	
	CHO	220 ± 52 (14)[e]	191 ± 11	3.7	34.1 ± 14.0 (7)[e]	Unchanged
3. F.G.	Fat	294 ± 27 (11)	212 ± 11	5.5	38.1 ± 5.0 (3)	
	CHO	596 ± 161 (18)[e]	217 ± 10	4.2	42.0 ± 3.3 (4)	Unchanged
4. T.N.	Fat	68 ± 10 (10)	216 ± 7	7.4	16.5 ± 3.6 (2)	
	CHO	218 ± 32 (18)[e]	260 ± 18	7.1	23.5 ± 3.2 (3)	Flattened
5. A.G.	Fat[f]	335 ± 47 (18)	184 ± 17	6.9	63.9 ± 1.6 (3)	
	Fat[g]	564 ± 82 (10)	224 ± 12	7.2		Unchanged
	CHO	796 ± 72 (11)[e]	254 ± 11[e]	6.6	87.9 ± 10.2 (3)[d]	Flattened
6. D.B.	Fat	190 ± 13 (9)	170 ± 8			
	CHO	426 ± 46 (9)	249 ± 19[e]			Flattened
7. R.B.	Fat[f]	66 ± 9 (9)	222 ± 10	3.2	19.9(1)	
	Fat[g]	98 ± 15 (11)	301 ± 30	4.2		Unchanged
	CHO	208 ± 36 (12)[e]	307 ± 21[e]	4.4	31.8 ± 8.6 (3)	Flattened
8. R.T.	Fat	1164 ± 72 (5)	237 ± 9			
	CHO	2048 ± 74 (6)[e]	337 ± 40[e]			Flattened
9. R.H.	Fat	263 ± 36 (15)	200 ± 15			
	CHO	624 ± 10 (18)[e]	354 ± 31[e]			Flattened
10. H.S.	Fat	854 ± 244 (15)	366 ± 14	8.6		
	CHO	1884 ± 143 (12)[e]	552 ± 47[e]	9.9		Flattened

[a] Values represent plasma levels during the metabolic steady state and are exclusive of data obtained during the transition from one feeding regimen to the next. Data in parentheses indicate the number of observations made.
[b] Mean of 2 analyses, 1 week apart in each dietary period.
[c] $P < 0.05$.
[d] $P < 0.025$.
[e] $P < 0.01$.
[f] 45% safflower oil.
[g] 45% olive oil.

then suspended in sterile 1.5% ethanolic saline for infusion (usual dose 100 μCi). Radioactivity was measured in a Packard TriCarb liquid scintillation counter (Model 3003) using external standard for automated quench corrections (Packard Instrument Co., Downers Grove, Ill.).

Chemical analyses

Concentrations of plasma cholesterol (16), triglycerides (17), and uric acid (18) were measured by autoanalyzer methods (Technicon Instruments Corp., Tarrytown, N.Y.); bleedings were twice weekly. Plasma squalene levels were measured by Dr. George Liu, The Rockefeller University (19). Plasma lipoproteins were separated by agarose gel electrophoresis (20) or by analytical ultracentrifugation (21).

Stool collections were continuous, usually as 4-day pooled specimens. Fecal steroids, chromic oxide, and plant sterols were determined by previously described methods (15, 22, 23). In calculations of daily cholesterol and bile acid synthesis rates, the appropriate corrections for neutral sterol losses and variations in fecal flow were used. With the onset of each new diet, Carmine Red was administered as a marker and a new stool collection period was started with the first appearance of dye in the feces.

Adipose tissue was aspirated from the buttocks and adipocytes were isolated after collagenase dispersion. Total adipocyte cholesterol concentration was determined by GLC after saponification and extraction (24).

Statistics

Statistics were performed by Olivetti computer using Student's t test for paired data, linear regression analysis, and the F test (25).

RESULTS

Plasma lipids

In all 10 patients plasma triglycerides increased on the high-carbohydrate diets (**Table 2**). This increase to a new but higher steady state level was achieved in 2–4 weeks and ranged from 76 to 238%. The increase in triglycerides was

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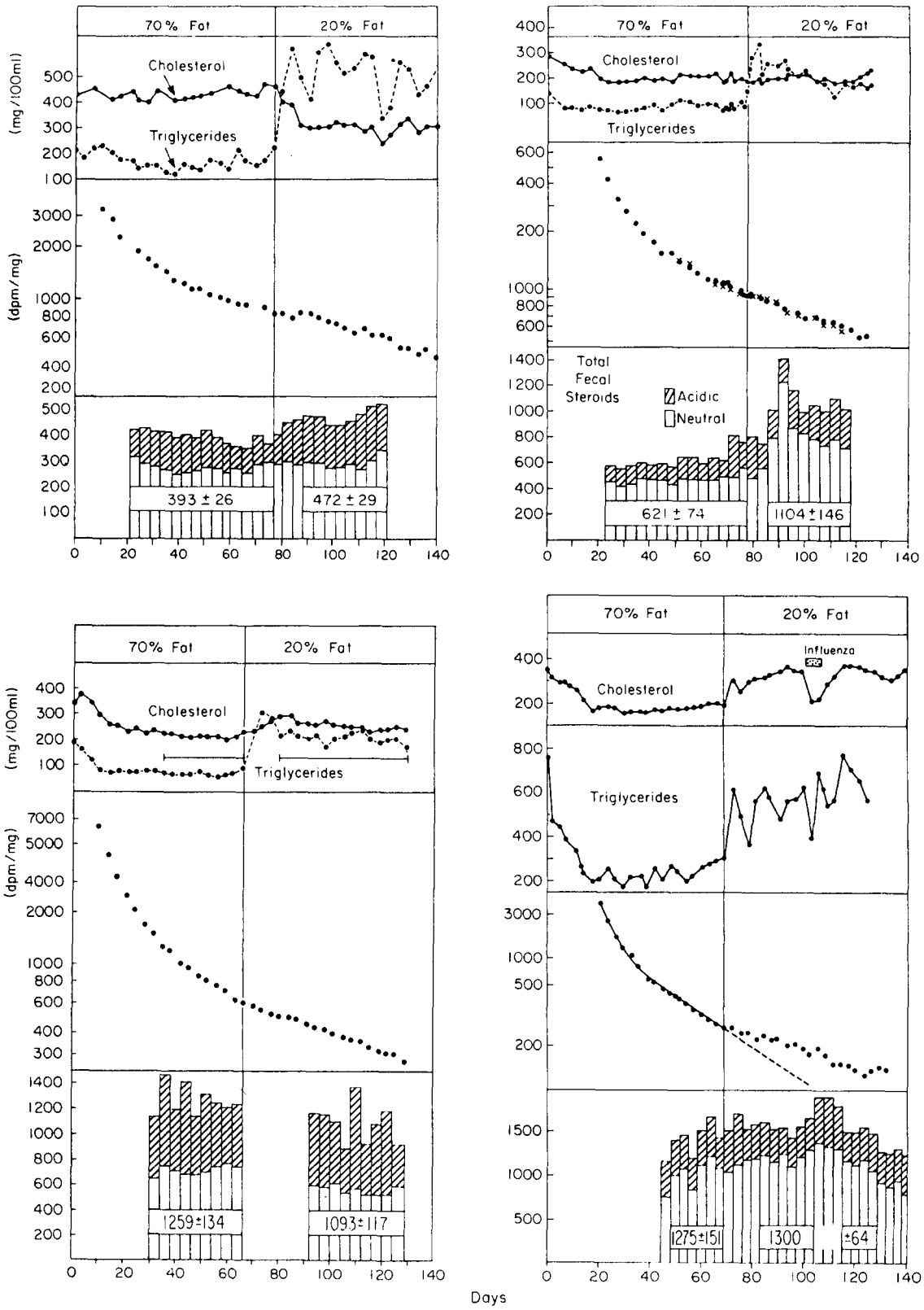

Fig. 1. Cholesterol balance data in Patients 1, 2, 3 and 9 (left to right, top to bottom, respectively). Diets contained varying amounts of fat and dextrose according to Table 1. The upper, middle and lower panels illustrate plasma lipid concentrations, plasma cholesterol specific activities and fecal acidic plus neutral steroid excretions, respectively. Fecal neutral steroid specific activities are denoted by x's in Patient 2. The total fecal steroid excretion is given as mg/day. Numbers in boxes represent mean ± S.D. Only Patient 9 had a significant flattening of the plasma die-away curve after carbohydrate exchange.

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TABLE 3. Percent distribution of plasma lipoproteins in patient no. 5

$F_{1.20}$[a]	185–802	61–185	44–61	16–44	0–6
S_F[b]	100–400	20–100	12–20	0–12	HDL_2 + HDL_3
Diet:					
45% Safflower	25	25	21	16	13
45% Olive	22	23	21	18	16
Fat-Free	32	30	26	8	4

[a] $F_{1.20}$ refers to flotation rates at d = 1.200, NaBr medium.
[b] S_F refers to estimated flotation rates at d = 1.200, NaCl medium as determined by del Gatto, et al. (21).

not correlated significantly with the increase in plasma cholesterol; as seen in patients 1–3, cholesterol remained unchanged or decreased as glycerides rose. Thus, plasma cholesterol rose in seven patients, remained unchanged in two, and decreased markedly in one.

Plasma lipoproteins

Agarose gel electrophoresis of fasting plasma was performed in each dietary period. In five patients studied the expected increase in pre-beta bands was observed during carbohydrate induction, and none developed fasting chylomicronemia. In each of the three dietary regimens for Patient 5, his plasma lipoproteins were separated by analytical ultracentrifugation; the data shown in **Table 3** are percentages of total lipoproteins found in each of five flotation classes. The major effect observed was a shift with carbohydrate induction from higher to lower density lipoproteins.

Plasma squalene

Determination of circulating squalene was performed on one to seven occasions in each dietary period on patients 1–5 and 7 (Table 2); the diets were squalene-free. In every patient, squalene levels were somewhat higher on the high-carbohydrate diets, regardless of changes in plasma cholesterol; changes were significant in three of six patients.

Plasma uric acid

Patient 10 had a past history of gouty arthritis and his plasma uric acid was slightly elevated (normal upper limit 7.5 mg%). Uric acid levels in the other nine patients were all within normal limits on these purine-free formula diets. In spite of carbohydrate-induction of plasma triglycerides in each case, uric acid levels remained unchanged (Table 2).

Cholesterol kinetics

Specific activities of plasma cholesterol were determined twice weekly in all patients after intravenous administration of a single dose of radioactive cholesterol. Representative results in 4 of the 10 patients are shown in **Fig. 1.** These charts were selected to illustrate the entire spectrum of responses that we observed among the 10 patients in plasma lipids, specific activity curves in plasma, and fecal steroid excretion.

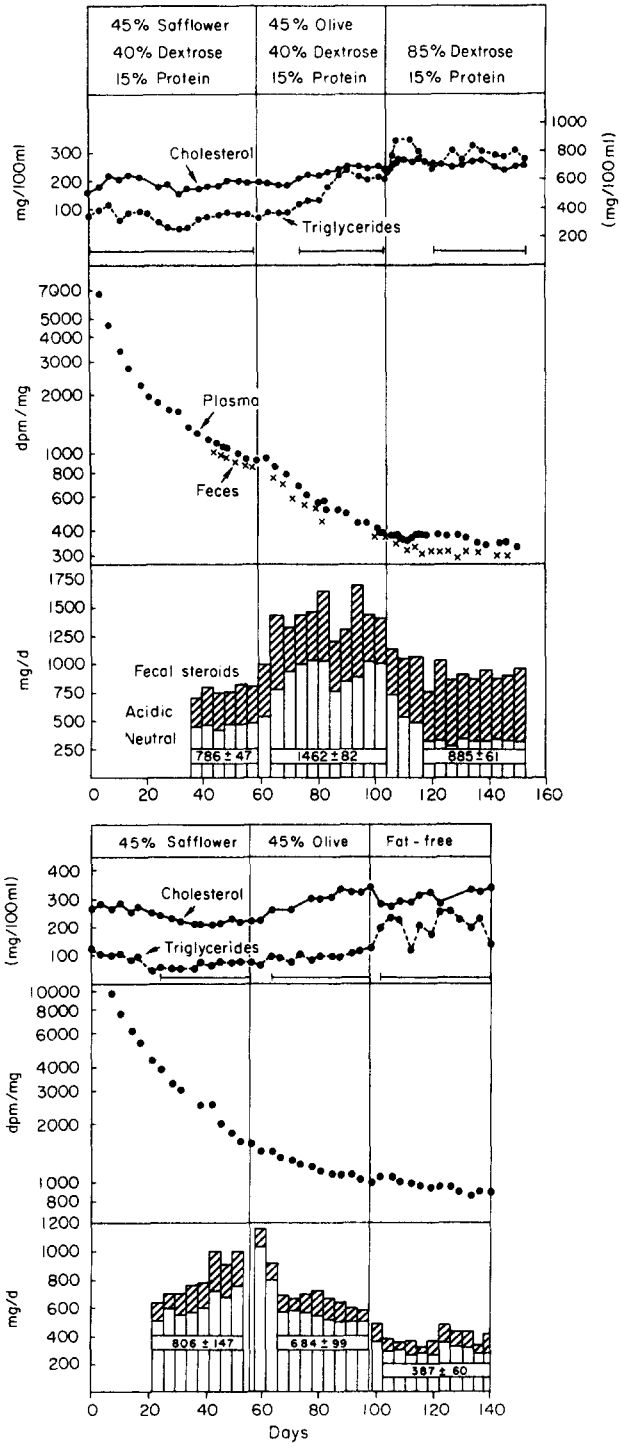

Fig. 2. Cholesterol balance data in Patients 5 (top) and 7 (bottom), respectively. Fecal neutral steroid specific activities are denoted by x's in Patient 5. This patient showed a marked increase in excretion after olive oil exchange for safflower oil which is, at present, unexplained. Patient 7 had the opposite response. Both patients showed a rise in plasma cholesterol with isocaloric exchange of either olive oil or dextrose for safflower oil.

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TABLE 4. Fecal steroid excretion during metabolic steady state on high-fat and high-carbohydrate regimens

Patient	High-Fat Diet			High-CHO Diet			Difference in Total Endogenous Fecal Steroid Excretion (CHO minus Fat)	
	Neutral Steroids	Acidic Steroids	Total Stercids	Neutral Steroids	Acidic Steroids	Total Steroids		
	mg/day; mean ± S.D. (n)						*mg/day*	*P*[a]
1. R.C.	269 ± 19 (14)	124 ± 20	393 ± 26	290 ± 22 (9)	181 ± 20	472 ± 29	+79	< 0.01
2. J.G.	469 ± 34 (14)	153 ± 54	621 ± 74	859 ± 169 (17)	246 ± 61	1104 ± 146	+483	< 0.001
3. F.G.	628 ± 14 (9)	192 ± 28	820 ± 31	718 ± 29 (7)	257 ± 43	974 ± 57	+154	< 0.001
4. T.N.	719 ± 39 (9)	541 ± 109	1259 ± 134	574 ± 109 (9)	519 ± 151	1093 ± 117	−266	< 0.01
5. A.G.	455 ± 21 (6)[b]	331 ± 39	786 ± 47	320 ± 19 (9)	565 ± 64	885 ± 61	+99	ns
6. R.B.	624 ± 89 (8)	183 ± 62	806 ± 147	292 ± 29 (9)	95 ± 40	387 ± 60	−419	< 0.001
7. D.B.	716 ± 71 (6)[b]	173 ± 45	889 ± 82	341 ± 30 (5)	155 ± 57	496 ± 49	−393	< 0.001
8. R.T.	974 ± 183 (7)	421 ± 28	1395 ± 174	350 ± 62 (6)	420 ± 59	770 ± 82	−625	< 0.001
9. R.H.	803 ± 88 (13)	472 ± 113	1275 ± 151	906 ± 63 (10)	396 ± 61	1302 ± 64	+25	ns
10. H.S.	670 ± 50 (9)	647 ± 95	1327 ± 116	718 ± 134 (10)	1148 ± 84	1866 ± 173	+539	< 0.001

[a] Student's paired *t* test between total fecal steroid excretions on each feeding regimen.
[b] 45% safflower oil diet.

All patients began the study on a high-fat diet and switched to a high-carbohydrate regimen after die-away curves became log-linear at 4–7 weeks. In the patients in whom plasma cholesterol levels rose with carbohydrate-induction, the die-away curves flattened significantly (*F* test statistics) (Table 2). Patient 1 showed a decrease in plasma cholesterol levels but no change in slope of her decay curve. Patients 2 and 3 had no change in plasma cholesterol or in specific activity slopes.

To determine if a flattened specific activity die-away curve was due to removal of dietary linoleic acid rather than to ingestion of carbohydrate, olive oil (rich in oleic acid) was isocalorically exchanged for safflower oil in patients 5 and 7 (**Fig. 2**); their specific activity curves showed no change in slope with this exchange of dietary fats. However, on exchange of carbohydrate for fat there was a significant flattening of the curves for both patients, suggesting a specific effect of dietary carbohydrate.

The specific activities of fecal neutral sterols also were measured in two of these studies, and results in patients 2 and 5 are illustrated in Figs. 1 and 2. In every case these specific activities were equal to or slightly lower than those of plasma cholesterol at the same point in time. However, the two sets of data declined in parallel, regardless of dietary changes, indicating a maintenance of isotopic equilibria between these two sterol compartments.

Fecal steroid excretion

Changes in excretion of total endogenous fecal steroids (endogenous neutral steroids, bile acids and their bacterial degradation products) were variable when carbohydrate was substituted for fat in the diet (**Table 4**). Patients 1–3, who failed to show an increased plasma cholesterol level, had significant increases in steroid excretion. By contrast, of the seven patients who experienced a rise in plasma cholesterol on the high-carbohydrate diet, only patient 10 showed a significant increase in total endogenous fecal steroid excretion. In two patients (5 and 9) there were no significant changes, while in the four others (4, 6, 7 and 8) there were significant decreases on the high-carbohydrate diet. During these metabolic steady states the variations in fecal steroid excretions were relatively constant in degree, and standard deviations were usually less than 10% of the mean.

The data in Table 4 are mean values for fecal steroid excretions obtained in the metabolic steady state and do not include the values obtained during the 1–2 week transition periods immediately following dietary changes. However, as shown in Figs. 1 and 2, there were no striking changes in fecal steroid excretions during the transition periods.

Changes in fecal neutral steroid excretion generally paralleled changes in fecal bile acid excretion. However, in Patients 7 and 8 the excretion of neutral steroids decreased but that of acidic steroids did not; in Patients 5 and 10 the rise in total endogenous fecal steroid excretion was due mainly to a striking increase in bile acid excretion.

Fecal β-sitosterol recovery

Dietary plant sterol intakes were held constant on the two dietary regimens. However, the recovery of β-sitosterol in feces (corrected for recovery of the nonabsorbable marker, Cr_2O_3) was lower on the high-carbohydrate diets in seven of eight patients, significantly so in five of those seven (**Table 5**). The mean recovery of β-sitosterol on the high-fat regimen was 97 ± 4% and on high-carbohydrate regimens 85 ± 10%; the difference was statistically significant.

Adipocyte cholesterol

Isolated adipocytes were obtained from seven patients during their various dietary regimens and were analyzed for cholesterol concentration per 100 mg lipid. Adipocyte cholesterol rose in six of seven patients on the high-carbohydrate diet; the increases reached statistical significance in three (**Table 6**). These increases occurred without necessary relation to plasma cholesterol changes. Patients 2 and 3, whose plasma levels did not rise, had increased concentrations of adipocyte cholesterol, whereas Patient 1, the only patient in

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TABLE 5. Fecal β-sitosterol recoveries[a]

Patient	High-Fat Diet	High-CHO Diet	P	Excretion of Total Endogenous Fecal Steroids[b]
	percentages as mean ± S.D. (n)			
1. R.C.	101 ± 13 (14)	85 ± 25 (9)	<0.05	increased
2. J.G.	90 ± 18 (14)	63 ± 15 (9)	<0.003	increased
3. F.G.	94 ± 8 (13)	80 ± 6 (8)	<0.001	increased
4. T.N.	103 ± 13 (8)	90 ± 14 (9)	<0.05	decreased
5. A.G.	94 ± 8 (6)	90 ± 7 (9)	ns	unchanged
6. R.B.	97 ± 13 (8)	98 ± 8 (9)	ns	decreased
9. R.H.	98 ± 12 (13)	85 ± 11 (10)	<0.025	unchanged
10. H.S.	100 ± 14 (9)	88 ± 18 (10)	ns	increased
Mean for 8 patients	97 ± 4	85 ± 10	<0.05	

[a] Corrected for chromic oxide recovery, i.e. (% recovery of β-sitosterol ÷ % recovery of Cr_2O_3) × 100.
[b] Significant change in total endogenous fecal excretion on high-CHO diet (from Table 4).

whom the plasma level fell significantly, was also the only patient with a slight decrease in adipocyte cholesterol.

DISCUSSION

Isocaloric substitution of dextrose for vegetable oil resulted either in changes of fecal steroid excretion, plasma cholesterol concentration, or both. Sterol balance results from the two olive oil exchanges will not be discussed because of the considerable differences in fatty acid saturation and squalene content between olive oil and the safflower, cottonseed or corn oils fed in the other 10 studies.

If the plasma cholesterol did not rise with carbohydrate induction of triglycerides, then fecal steroid excretion was invariably greater in the new "steady state". If the increase in triglycerides was accompanied by an increase in plasma cholesterol, then fecal excretion was unchanged or decreased. Whyte, Nestel, and Pryke (13) observed a higher plasma cholesterol in four healthy volunteers when a solid-food cholesterol-containing diet rich in sucrose was exchanged isocalorically for a diet rich in polyunsaturated fat. Although bile acid excretion rose on the sucrose diet, neutral sterol excretion fell. Thus, total sterol balance remained unchanged during the 2 weeks of study. Our observations agree in regard to carbohydrate-related increase of plasma cholesterol in 7 out of 10 hyperlipidemic patients, but bile acid excretion increased in only 5 of 10 and total sterol balance was significantly altered in 8 of 10 patients.

Increased excretion

An increased excretion of fecal steroids may be due to three factors: increased synthesis, increased tissue mobilization, or decreased absorption. The last possibility seems unlikely because all diets were essentially cholesterol-free. However, a decrease in reabsorption of endogenous biliary cholesterol might occur, which in turn could result in increased synthesis or tissue mobilization.

TABLE 6. Dietary effects on adipocyte cholesterol concentrations

Patient	Diet: High-Fat	Diet: High-CHO	P
	mg cholesterol/100 mg lipid mean ± SD (n)		
1.	0.20 ± 0.02 (7)	0.18 ± 0.01 (3)	<0.10
2.	0.20 ± 0.02 (5)	0.21 ± 0.01 (5)	<0.20
3.	0.16 ± 0.01 (7)	0.20 ± 0.02 (5)	<0.01
4.	0.20 ± 0.03 (5)	0.23 ± 0.05 (10)	<0.15
5.	0.15 ± 0.02 (5)	0.19 ± 0.02 (4)	<0.025
6.	0.17 ± 0.02 (7)	0.18 ± 0.01 (4)	<0.20
9.	0.16 ± 0.02 (6)	0.20 ± 0.03 (11)	<0.01

We feel that a slow mobilization of tissue cholesterol stores is unlikely for the following reasons: Adipose tissue is the single major site for cholesterol storage in man (24, 26). Actual measurements of adipose tissue cholesterol in these patients showed a small but significant rise in concentration (mg cholesterol per cell) on high-carbohydrate diets (Table 6). Only in Patient 1, where plasma cholesterol decreased by over 100 mg/100 ml, was there a significant decrease in adipocyte cholesterol concentration. In addition, increased fecal steroid excretion was sustained for periods up to 2 months and showed no sign of diminishing. In Patient 2, for example, the difference in excretion was almost 0.5 g/day, amounting to 22 g of cholesterol within a 44-day period. The total miscible cholesterol pool in this patient was estimated as less than 100 g, of which plasma and adipose tissue accounted for over 25 g. It is difficult to conceive of 22 g of cholesterol being lost in such a short period of time from a total available depot of 75 g.

Evidence favoring increased endogenous synthesis of cholesterol to account for increased excretion is found in an increased plasma squalene concentration on high-carbohydrate

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diets. Squalene is an obligatory precursor of sterols and in rats its plasma level correlates directly with hepatic cholesterol synthesis rates (27) as measured by radioisotopic acetate incorporation and sterol balance experiments. This evidence is indirect, however, and elevation of plasma squalene could also be explained by an increased concentration of VLDL which may serve as a squalene transport carrier (19, 28).

Nestel et al (29, 30) have reported an increased cholesterol ester turnover in man during carbohydrate-rich diets. Heimberg and Wilcox (31) have shown that, in a perfused rat liver preparation, an increased turnover of VLDL triglyceride is accompanied by increased VLDL-cholesterol and phospholipid secretion. They postulate that the latter two lipids are necessary for physical-chemical stability of a very low density lipoprotein. If this situation exists in man, then increased cholesterol synthesis may be a consequence of a primary carbohydrate stimulation of VLDL synthesis.

Decreased or unchanged excretion

If an increased steroid excretion does reflect a new steady state of increased hepatic cholesterol synthesis in Patients 1–3, then a decreased or unchanged excretion in Patients 4–9 might correspondingly imply similar responses in endogenous synthesis. It must be remembered however, that these excretion values represent data collected after plasma lipids and fecal steroids had reached relatively constant levels. A transition state of increased cholesterol synthesis could result in a retention of sterol in plasma. This might in turn feed back and inhibit the liver's capacity to continue its augmented cholesterogenesis. The net result of this inability to excrete plasma cholesterol would be an unchanged or even decreased fecal steroid excretion in the new steady state. The unique results in Patient 10 may represent an intermediate response in which the newly synthesized cholesterol is partially retained and partially excreted; alternatively, feedback inhibition of hepatic synthesis may be insufficient to prevent an overall increase in excretion. The increased adipocyte cholesterol concentrations reflected an overall retention of cholesterol within the body.

Thus, in all 10 patients carbohydrate feeding could initially result in an increased hepatic cholesterol synthesis. Whether this greater synthesis rate persists or not would depend on the disposition of the cholesterol product, i.e., retention in the circulation or excretion into the feces. Although the foregoing interpretation is attractive, it is equally possible that a heterogenous group of patients respond heterogenously. If so, then in those patients responding to carbohydrate exchange with decreased cholesterol synthesis it becomes necessary to invoke a mobilization of cholesterol from tissue stores to account for the paradoxical rise in plasma cholesterol.

Isotope kinetics

The specific activity of fecal neutral sterol was essentially identical to plasma cholesterol specific activity on both diets (Figs. 1 and 2). This must be true if an isotopic steady state applies between plasma and intestinal contents. This finding also eliminates the possibility of a carbohydrate induction of intestinal cholesterogenesis in which the newly synthesized cholesterol is directly excreted with the feces. If such were the case, fecal neutral sterol specific activity would have been lower than plasma.

In all patients with a rise in plasma cholesterol on high-carbohydrate diets, the die-away curve of plasma cholesterol specific activity flattened significantly. Such an event might be due to either decreased synthesis or increased exchange of plasma cholesterol with some slowly turning-over pool of body cholesterol.

Adipocyte cholesterol. Compartmental analysis of specific activity curves showed adipocyte cholesterol to be in the slowly turning-over Pool 3 of body cholesterol. Specific activities for this tissue were consistently 2–3 times greater than plasma at the time dietary interventions were performed (24). This cholesterol pool could exchange with VLDL cholesterol at a faster rate than with LDL or HDL cholesterol. Thus, an increased plasma VLDL concentration as demonstrated in these patients during high-carbohydrate feeding might result in "hotter" adipocyte cholesterol exchanging for "colder" plasma cholesterol and hence a flattened plasma specific activity slope.

Carbohydrate specificity. We have not employed starch or sucrose exchanges in these studies and cannot therefore comment on the specificity of dextrose as the responsible nutrient for the flattening of die-away curves. The observation in Patient 4 (Fig. 2) that isocaloric exchange of olive oil (iodine value = 90) for safflower oil (I.V. = 140) does not result in such a flattening suggests, however, that mere removal of polyunsaturated fat will not produce this response. The enlarged lipoprotein particle size (Table 3) on high-carbohydrate but not on olive oil feeding may be important in cholesterol exchange between adipose tissue and plasma.

Sitosterol recovery

Beta-sitosterol is essentially unabsorbed in man, but may be destroyed or converted by intestinal microorganisms to some unrecoverable form (32). This process has not been seen in patients eating solid food diets and may be a function of bacterial flora changes induced by liquid formula diets. Our finding that β-sitosterol recovery is reduced on high-carbohydrate formula feeding may offer an experimental model for the study of this interesting phenomenon.

Uric acid

Elevations of plasma uric acid are known to occur with greater frequency in patients with hypertriglyceridemia (33–35). Our observation that an acute carbohydrate-induction of triglycerides on a fixed protein, purine-free diet did not produce a concomitant rise in uric acid suggests that diet alone is not responsible for this frequently observed clinical association.

SUMMARY

Isocaloric exchange of dextrose for polyunsaturated chole-

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sterol-free vegetable oil uniformly increased plasma triglycerides in all 10 patients studied. Plasma cholesterol rose in only 7, and this was associated with decreased fecal cholesterol excretion. In the remaining 3, excretion increased significantly without a rise in plasma cholesterol. We have offered one of several possible hypotheses to explain these observations: dietary carbohydrate stimulates cholesterol synthesis, but the body's ability to clear cholesterol eventually determines the plasma cholesterol level. To test this hypothesis we must utilize a reliable non-invasive method for measuring cholesterol synthesis in the unsteady state following dietary intervention (36).

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