The effect of cholesterol feeding on bile acid kinetics and biliary lipids in normolipidemic and hypertriglyceridemic subjects

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Abstract Six normolipidemic and six hypertriglyceridemic subjects were studied. The investigations were conducted before and after the basal diet (cholesterol intake about 0.8 mmol/day) was replaced by a cholesterol-rich diet (cholesterol intake about 4 mmol/day). Irrespective of the type of diet, the combined formation of cholic acid (C) and chenodeoxycholic acid (CD) was about two times higher in the hyperlipoproteinemic (mostly type IV) than the normolipidemic subjects. With the cholesterol-rich diet, the total plasma cholesterol increased in all normolipidemic and in four hyperlipidemic patients. Although total bile acid formation remained constant, there were several indications that an augmented intake of dietary cholesterol influenced bile acid metabolism. The pool size of CD increased in all but one normolipidemic subject. This group also displayed a decrease in the C/CD ratio of the bile acids produced and in the C/CD ratio of the bile acids in duodenal bile. The latter finding was also encountered in the hyperlipoproteinemic patients. On the basis of these and other data, it is suggested that the pattern of the bile acids synthesized may roughly reflect the degree of hepatic cholesterogenesis. Cholesterol feeding had no consistent effects on the molar cholesterol concentration in duodenal bile.—**Andersén, E., and K. Hellström.** The effect of cholesterol feeding on bile acid kinetics and biliary lipids in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res.* 1979. **20:** 1020–1027.

Supplementary key words cholic acid · chenodeoxycholic acid · hyperlipoproteinemia.

Several lines of evidence suggest that the mechanisms regulating bile acid formation may be somewhat different for cholic acid (C) and chenodeoxycholic acid (CD). Studies in rats indicate that cholic acid, to a higher extent than chenodeoxycholic acid, originates from newly synthesized liver cholesterol (1). In accordance with this observation, the biosynthesis of cholic acid showed a proportionally higher increase than that of chenodeoxycholic acid upon stimulation of hepatic cholesterogenesis by administration of a bile acid-sequestering agent both in normolipidemic subjects (2) and in patients with the type II lipoprotein pattern (3). If these findings have a general implication, the inhibition of hepatic cholesterogenesis by cholesterol feeding (4) may influence the pattern of the two primary bile acids produced. This hypothesis was tested in the current study, in which the kinetics of cholic and chenodeoxycholic acids were studied in normo- and hyperlipidemic subjects before and during intake of a cholesterol-rich diet.

MATERIAL AND METHODS

The subjects in the study comprised six normolipidemic female members of the hospital staff and six hyperlipidemic patients (**Table 1**). On the basis of numbers of previous analysis, subject 5 was considered normolipidemic although she was slightly hypercholesterolemic at the time of this study. The ages ranged between 45 and 62 years. All females except subjects 4 and 7 were postmenopausal. As judged by physical and laboratory investigations, the normal subjects were healthy. Two of the patients suffered from ischemic heart disease and two (subjects 8 and 9) had previously undergone cholecystectomy because of cholesterol gallstone disease. There was no evidence of intestinal, hepatic or renal disease, heart failure, thyroid dysfunction or addiction to alcohol and narcotics in any of the subjects. None of the subjects had been treated with drugs or diets known to interfere with serum lipoproteins during the months preceding this investigation. Five of the six hyperlipidemic patients were overweight (relative body weight higher than 120%) (Table 1). All subjects were informed of the nature, purpose, and risks involved in the study before giving their informed consent.

Abbreviations: C, cholic acid; CD, chenodeoxycholic acid; GLC, gas-liquid chromatography; HDL, high density lipoprotein; HLP, hyperlipoproteinemia; LDL, low density lipoprotein; VLDL, very low density lipoprotein; FTR, fractional turnover rate.

TABLE 1. Basal data and blood lipids before (A) and during (B) intake of cholesterol-rich diet

Subject	Age Sex	Type of HLP[a]	Rel Body Weight[b]	Serum Cholesterol	Serum Triglyceride
			%	*mmol/l*[c]	*mmol/l*[c]
1. MS	58 F	N	91	A 5.3 ± 0.11[d]	1.1 ± 0.08[d]
				B 6.2 ± 0.11	1.3 ± 0.21
2. BB	55 F	N	87	A 6.2 ± 0.13	1.0 ± 0.07
				B 7.7 ± 0.12	1.0 ± 0.03
3. MH	51 F	N	92	A 5.1 ± 0.12	0.7 ± 0.03
				B 5.6 ± 0.05	0.8 ± 0.03
4. RL	49 F	N	119	A 5.4 ± 0.23	1.2 ± 0.17
				B 5.9 ± 0.11	1.1 ± 0.11
5. TL	53 F	N	111	A 7.3 ± 0.22	1.2 ± 0.31
				B 7.8 ± 0.35	1.1 ± 0.13
6. EC	61 F	N	84	A 5.4 ± 0.05	0.8 ± 0.07
				B 6.6 ± 0.19	1.1 ± 0.15
7. SK	45 F	IIb	137	A 7.9 ± 0.36	3.1 ± 0.10
				B 8.8 ± 0.49	4.2 ± 0.36
8. SA	61 F	IV	143	A 6.5 ± 0.05	6.3 ± 0.32
				B 6.0 ± 0.08	6.2 ± 0.55
9. SE	53 F	IV	131	A 6.0 ± 0.17	5.3 ± 0.17
				B 6.7 ± 0.13	5.7 ± 0.26
10. EP	62 M	IV	123	A 5.6 ± 0.10	4.3 ± 0.34
				B 6.7 ± 0.15	7.0 ± 0.38
11. ÅE	53 M	IV	108	A 5.5 ± 0.18	2.4 ± 0.11
				B 5.6 ± 0.28	1.8 ± 0.17
12. GK	58 M	IV	153	A 4.3 ± 0.75	4.6 ± 0.18
				B 6.0 ± 0.13	5.8 ± 0.33
Normal				A 5.8 ± 0.34	1.0 ± 0.09
				B 6.6 ± 0.38[e]	1.1 ± 0.07
Type IIb + IV				A 6.0 ± 0.49	4.3 ± 0.58
				B 6.6 ± 0.47	5.1 ± 0.76
Type IV				A 5.6 ± 0.37	4.6 ± 0.64
				B 6.2 ± 0.22	5.3 ± 0.90

[a] Abbreviations: HLP, hyperlipoproteinemia; F, female; M, male.
[b] Relative body weight: weight (kg)/length (cm) − 100 × 100%.
[c] To convert mmol to mg/dl multiply cholesterol concentrations by 38.7 and triglyceride concentrations by 0.1 × mol wt of triglyceride (e.g., 88.5 for triolein).
[d] Means ± SEM of 4 or 5 analyses.
[e] Significantly different from corresponding value on basal diet $P < 0.01$.

Experimental protocol

Bile acid kinetics were studied twice in each subject, and the patients were hospitalized during each experimental period. The normolipidemic subjects continued with work that was essentially sedentary. For one week before and during the first turnover study all subjects were given a weight-maintaining diet prepared from solid food. Of the calories 36, 44 and 20% were supplied as fat, carbohydrate, and protein, respectively. The fat contained mainly saturated fatty acids. Prior to the experiments the subjects were interviewed about their dietary habits by a dietician and their energy requirements were estimated. Minor adjustments of the food intake had to be made during the study to keep the body weight constant. For 2 weeks before and during the second study the fat content in the basal diet was reduced and isocalorically compensated for by the fat in five eggs, supplied as a plain omelette. With this procedure the daily cholesterol intake during the first and the second studies averaged 0.8 and 4.0 mmol, respectively, in the normolipidemic subjects. The corresponding figures for the hyperlipidemic patients were 0.9 and 4.1 mmol, respectively. Body weight and the plasma lipid levels were measured repeatedly during the studies.

Methods

Cholesterol and triglycerides in serum were measured using a discrete multi-channel analyzer (Auto-

chemist, LKB Clinicon, Bromma, Sweden). Lipoprotein phenotyping was performed according to WHO recommendation (5) as described earlier (6). Serum samples from some of the subjects (subjects 3–7, 11, 12) were also analyzed with regard to individual lipoproteins. Venous serum was separated by low speed centrifugation, 5% EDTA was added to a final concentration of 0.05%, and the samples were left at 4°C overnight. When present, the thin layer of chylomicrons was removed by aspiration. Serum lipoproteins were fractionated by ultracentrifugation (Damon/IEC ultracentrifuge model B60, Needham, MA) following procedures described by Carlson (7). The LDL (low density lipoprotein) fraction was separated from HDL (high density lipoprotein) by precipitation of the former with manganese heparin (8). In these fractions cholesterol was determined as described by Hanel and Dam (9) and triglycerides were determined with a kit, obtained from Boehringer-Mannheim GmbH, West Germany.

Bile acid kinetics were determined after oral administration of the sodium salts of [^{14}C]cholic acid (4 μCi) and [^{14}C]chenodeoxycholic acid (4 μCi) in the morning before breakfast. Four samples of duodenal bile were collected at 2–4 day intervals into ice-chilled tubes. Cholecystokinin was administered intravenously and 5–10 ml of concentrated duodenal bile was obtained through a thin polyvinyl tube. The bile samples were hydrolyzed with 1 M KOH in closed steel tubes for 12 hr at 110°C. After acidification the bile acids were extracted with ethyl ether, and their methyl esters (prepared by treatment with diazomethane) were subsequently separated by thin-layer chromatography. One aliquot of the fractions containing C and CD was analyzed by gas–liquid chromatography (GLC) on a 3% SE–30 column after preparation of their trimethylsilyl derivatives (10). Injection port heaters and detectors were operated at 250°C with column temperature of 220°C and carrier gas flow rates of 30 ml/min. Another aliquot of the extract was analyzed for radioactivity by liquid scintillation counting. On the basis of the specific radioactive curve, the pool size, synthesis and fractional turnover rate (FTR) of C and CD were determined as described by Lindstedt (11). The correlation coefficients for the specific activity decay curves of C and CD averaged 0.996 ± 0.001 and 0.997 ± 0.001, respectively, during the basal study, and 0.994 ± 0.001 and 0.993 ± 0.001 when the subjects were fed the cholesterol-rich diet.

The duodenal bile was hydrolyzed as described above and the bile acids were extracted. The trifluoroacetate derivatives of the bile acid methyl esters were prepared and analyzed by GLC on a 3% QF-1 column. Other aliquots of duodenal bile samples were used for the determination of total bile acid content (12), cholesterol (9), and phospholipids (13). Lipid composition in bile was expressed as mole per cent bile acids, cholesterol, and phospholipids (14). Cholesterol saturation of bile in noncholecystectomized patients was calculated according to Carey and Small (15) using the solubility line for a biliary lipid concentration of 10 g/dl (16). Dietary cholesterol was determined by GLC on a 3% SE–30 column, the bulk of dietary triglyceride being eliminated by silicic acid column chromatography (17). Detailed descriptions of the methods used are to be found in previous papers (6, 18).

Material

[24-^{14}C]Cholic acid (138 μCi/mg) and [24-^{14}C]-chenodeoxycholic acid (138 μCi/mg) were obtained from New England Nuclear Corp., Boston, MA. The radiochemical purity of the labeled bile acids was ascertained by radioautography of thin-layer chromatograms. A purified 3 α-hydroxysteroid dehydrogenase (Sterognost 3α) was purchased from Nyegaard & Co, A1S, Oslo, Norway. Cholecystokinin was obtained from the Gastrointestinal Hormone Research Group, Department of Chemistry, Karolinska Institute, Stockholm, Sweden.

Statistical analysis

Data are presented as means ± SEM. The significance of differences was evaluated by Student's paired t test (19).

RESULTS

Serum lipids

Analyses (4–5 per subject), performed when the new plateau level was reached after 2 weeks, demonstrated that the intake of the cholesterol-rich diets resulted in an elevation of the total serum cholesterol concentration in all normolipidemic subjects (Table 1). These changes, as evidenced by studies in four of the normolipidemic subjects, were associated with a 10% increase of cholesterol in the LDL fraction. VLDL decreased and HDL cholesterol increased slightly in three of the subjects whereas subject 4 reacted in a somewhat different way (**Fig. 1.**). Increased cholesterol levels after the change of diets were also found in four of the six hyperlipoproteinemic patients. No consistent changes were observed in the LDL-cholesterol in the three patients studied in this respect (patients no. 7, 11 and 12). The serum triglyceride levels

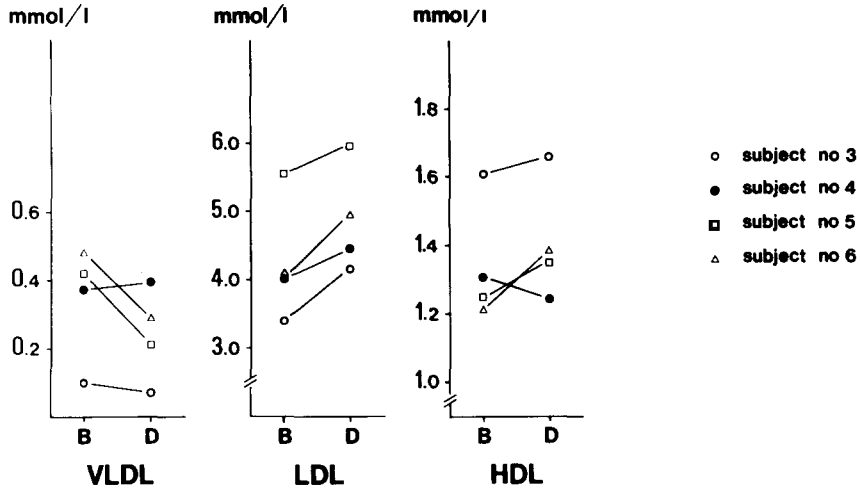

Fig. 1. The cholesterol concentration in VLDL, LDL, and HDL before (B) and during (D) cholesterol feeding.

showed no variations that could be related to an increased intake of cholesterol.

Bile acid kinetics

When studied under basal conditions, the normolipidemic subjects displayed a pool size, synthesis, and FTR of C that averaged 2.34 mmol, 0.70 mmol/day and 0.32 day^{-1}, respectively (**Table 2**). None of these parameters showed significant changes upon the shift from the basal to the cholesterol-rich diet. Similarly, the synthesis and FTR of CD remained the same but the CD pool size increased in five out of the six normolipidemic subjects, on an average from 1.53 ± 0.24 to 1.87 ± 0.31 mmol ($P < 0.05$). As a more sensitive index of a possible alteration in individual bile acid metabolism, the C/CD ratio was calculated for the bile acid pool size and synthesis (Table 2). After the change of diet, the ratio of C pool size to CD pool size decreased in four subjects and remained unchanged in two. The C/CD ratio for the bile acids synthesized was reduced in all subjects; the means during the first and second dietary periods were 1.87 ± 0.16 and 1.59 ± 0.09, respectively ($P < 0.05$). Expressed in another way, the mean percentage contribution of C to the total amount of bile acids synthesized was 64.2% when the subjects were fed the basal diet and 61.8% when fed the cholesterol-rich diet. The change of diet was also reflected in the composition of the three main bile acids in duodenal bile. In keeping with the finding mentioned above, the C/CD ratio showed a significant fall during the second dietary period (**Table 3**).

The bile acid kinetics in the hyperlipoproteinemic patients differed in several respects from the corresponding parameters in the normolipidemic subjects. While the pool sizes of C and CD showed no significant differences in the two groups, the total bile acid formation was about twice as high in the hyperlipoproteinemic as in the normolipidemic subjects, irrespective of the type of diet. Another difference was that the FTR of C was higher in the former than in the latter group. Mainly due to a large pool size of CD in two patients (subjects 8 and 10), the mean ratio between C and CD pool sizes was smaller in the hyperlipoproteinemic patients. The C/CD ratio for bile acid synthesis showed the same values as found for the normolipidemic subjects. The change from the basal to the cholesterol-rich diet had no significant effect on the bile acid kinetics in the patients, but the composition of the bile acids in bile was altered in a manner similar to that observed for the normolipidemic subjects (Tables 2 and 3).

Biliary lipids

When studied under basal conditions, the molar cholesterol concentration averaged 6.3 ± 0.77 mol% in the normolipidemic and 10.5 ± 0.89 mol% in the hyperlipidemic subjects (**Table 4**). No significant changes were observed during cholesterol feeding, the mean values for the molar cholesterol level being 6.0 ± 0.80 and 9.5 ± 0.91% in the normo- and hyperlipidemic subjects, respectively. Cholesterol saturation (calculated for noncholecystectomized patients) did not change in a significant manner upon the shift of diets.

DISCUSSION

Liver cholesterol is incorporated into serum lipoproteins, excreted in the bile, or converted to bile

TABLE 2. Bile acid kinetics when the subjects were fed basal (A) and cholesterol-rich (B) diets

Subject	Type of HLP	Type of Diet	Cholic Acid (C)[a] Pool Size	Cholic Acid (C)[a] Synthesis	Cholic Acid (C)[a] FTR	Chenodeoxycholic Acid (CD)[a] Pool Size	Chenodeoxycholic Acid (CD)[a] Synthesis	Chenodeoxycholic Acid (CD)[a] FTR	C + CD Pool Size	C + CD Synthesis	C/CD Pool Size	C/CD Synthesis
			mmol	*mmol/day*	*day*$^{-1}$	*mmol*	*mmol/day*	*day*$^{-1}$	*mmol*	*mmol/day*		
1.	N	A	2.74	0.63	0.23	2.64	0.46	0.17	5.38	1.09	1.04	1.37
		B	3.36	0.49	0.15	3.20	0.42	0.13	6.56	0.91	1.05	1.17
2.	N	A	2.77	0.66	0.24	1.17	0.26	0.22	3.94	0.92	2.37	2.54
		B	1.15	0.53	0.46	1.00	0.30	0.30	2.15	0.83	1.15	1.77
3.	N	A	2.36	0.75	0.32	1.40	0.40	0.29	3.76	1.15	1.69	1.88
		B	2.01	0.56	0.28	1.88	0.33	0.17	3.89	0.89	1.07	1.70
4.	N	A	1.58	0.80	0.51	1.03	0.41	0.40	2.61	1.21	1.53	1.95
		B	1.59	1.15	0.72	1.37	0.67	0.49	2.96	1.82	1.16	1.72
5.	N	A	2.95	0.82	0.28	1.74	0.49	0.28	4.69	1.31	1.70	1.67
		B	2.50	0.79	0.31	2.04	0.49	0.24	4.54	1.28	1.23	1.61
6.	N	A	1.66	0.55	0.33	1.17	0.30	0.26	2.83	0.85	1.42	1.83
		B	2.40	0.54	0.23	1.72	0.35	0.20	4.12	0.89	1.40	1.54
7.	IIb	A	1.89	1.08	0.57	1.95	0.87	0.45	3.84	1.95	0.97	1.24
		B	1.94	1.10	0.57	2.26	0.77	0.34	4.20	1.87	0.86	1.43
8.	IV	A	2.46	2.28	0.93	3.27	1.21	0.37	5.73	3.49	0.75	1.88
		B	3.35	2.15	0.64	4.65	0.91	0.20	8.00	3.06	0.72	2.36
9.	IV	A	1.79	1.24	0.69	1.45	0.66	0.46	3.24	1.90	1.23	1.88
		B	0.94	0.74	0.79	0.91	0.51	0.56	1.85	1.25	1.03	1.45
10.	IV	A	2.93	2.12	0.72	5.08	1.07	0.21	8.01	3.19	0.58	1.98
		B	3.63	2.37	0.65	5.73	1.22	0.21	9.36	3.59	0.63	1.94
11.	IV	A	1.85	1.71	0.92	1.55	1.04	0.67	3.40	2.75	1.19	1.64
		B	2.12	1.86	0.88	1.95	1.02	0.52	4.07	2.88	1.09	1.82
12.	IV	A	1.78	0.62	0.35	2.02	0.56	0.28	3.80	1.18	0.88	1.11
		B	1.78	0.78	0.44	1.77	0.69	0.39	3.55	1.47	1.01	1.13
Mean	N	A	2.34	0.70	0.32	1.53	0.39	0.27	3.87	1.09	1.63	1.87
SEM			0.24	0.04	0.04	0.24	0.04	0.03	0.43	0.07	0.18	0.16
		B	2.17	0.68	0.36	1.87[b]	0.43	0.26	4.04	1.10	1.18	1.59[b]
			0.31	0.11	0.08	0.31	0.06	0.05	0.62	0.16	0.05	0.09
Mean	IIb	A	2.12	1.51[c]	0.70[d]	2.55	0.90[e]	0.41	4.67	2.41[d]	0.93[d]	1.62
SEM	+ IV		0.19	0.26	0.09	0.57	0.10	0.07	0.76	0.36	0.10	0.15
		B	2.29	1.50[c]	0.66[c]	2.88	0.85[d]	0.37	5.17	2.35[c]	0.89[c]	1.69
			0.41	0.29	0.06	0.77	0.10	0.06	1.17	0.39	0.08	0.18
Mean	IV	A	2.16	1.59[c]	0.72[d]	2.67	0.91[d]	0.40	4.84	2.50[d]	0.93[c]	1.70
SEM			0.23	0.30	0.11	0.68	0.13	0.08	0.91	0.43	0.13	0.16
		B	2.36	1.58[c]	0.68[c]	3.00	0.87[d]	0.38	5.37	2.45[c]	0.90[c]	1.74
			0.50	0.34	0.07	0.93	0.12	0.08	1.42	0.46	0.09	0.21

[a] The molecular weight of cholic acid is 408.4; that of chenodeoxycholic acid is 392.0.
[b] Significantly different from corresponding value on basal diet ($P < 0.05$).
[c] Significantly different from corresponding value in normolipidemic subjects ($0.01 < P < 0.05$).
[d] Significantly different from corresponding value in normolipidemic subjects ($0.001 < P < 0.01$).
[e] Significantly different from corresponding value in normolipidemic subjects ($P < 0.001$).

acids. The outflow of cholesterol from the liver is balanced by hepatic cholesterogenesis and by an inflow of lipoprotein cholesterol. The mechanisms responsible for the overall regulation of these processes are incompletely known. Beyond doubt the formation of bile acids is monitored by a negative feed-back control, triggered by the amounts of bile acids reaching the liver via the portal vein (20). Bile acid biosynthesis also seems to be influenced by mechanisms regulating VLDL formation: an augmented production of VLDL is associated with an enhanced degradation of cholesterol to bile acids (21). However, the relationship between the metabolism of VLDL, cholesterol and bile acids may be bidirectional, as stimulation of bile acid formation by cholestyramine results in an accelerated synthesis of both hepatic cholesterol (20) and VLDL (22).

Part of the lipoprotein cholesterol delivered to the liver is incorporated in chylomicron remnants. The uptake of such particles by the liver is known to regulate hepatic cholesterogenesis by feed-back inhibition (23). Several lines of evidence indicate that the rate of cholesterol synthesis in the liver is reflected in the pattern of the two primary bile acids produced. When cholestyramine is administered to normolipidemic subjects, the formation of C in general acelerates more than that of CD (2). Moreover, in most instances the C/CD ratio of the bile acids synthesized is higher in type IV hyperlipoproteinemia than in type II (6), two conditions that differ with regard to net steroid balance (18). In view of these findings, it appeared of interest to study the effect of cholesterol feeding on the kinetics of the two primary bile acids C and CD.

In evaluating the present results it is necessary to consider the experimental conditions used. The technique of measuring bile acid turnover requires steady state conditions, i.e., synthesis must equal excretion and the pool size has to be constant during the experimental period. To meet these requirements the patients were given a standardized diet before and during each experimental period. All patients were on the cholesterol-rich diet for at least 2 weeks prior to the second turnover study. At the time of that study the serum lipids had reached a new plateau level. The linear slopes recorded for the specific activity of the bile acids in duodenal bile indicated that bile acid metabolism had also reached a new steady state.

In contrast to the findings of Den Besten, Connor, and Bell (24), but confirming the reports of Sarles (25) and Dam et al. (26), the shift of diets had no consistent effect on cholesterol saturation in bile. As an indication of the augmented uptake of dietary cholesterol, the total serum and LDL cholesterol were elevated in the normolipidemic subjects. This response to the cholesterol-rich diet was less pronounced and furthermore was inconsistent in the patients with HLP. In accordance with the observation of Quintao, Grundy, and Ahrens (27) and Nestel and Poyser (28), the feeding of cholesterol had no effect on the overall degradation of cholesterol to bile acids. However, the reduced C/CD ratio of the bile acids in duodenal bile indicates that cholesterol feeding influenced bile acid metabolism in normo- as well as hyperlipoproteinemic subjects. This effect in the former group was also reflected by a significant elevation of the CD pool size and, more importantly, in a reduction of the C/CD ratio of the bile acids synthesized. In accordance with previous findings (6), the C/CD ratios of the bile acid pool often differed from the corresponding ratios observed for the bile acids recovered in bile. This discrepancy might in part be explained by all the evidence indicating that C and CD circulate through the enterohepatic circulation at different rates (29, 30).

TABLE 3. C:CD:D ratio in duodenal bile before (A) and during (B) intake of a cholesterol-rich diet

Subject Group	Type of Diet	C	:	CD	:	D[a]
Normal						
Mean	A	2.30		1		1.58
SEM		0.29				0.44
	B	1.94[c]		1		1.87
		0.30				0.43
Type IIb and IV						
Mean	A	2.07		1		2.18
SEM		0.51				1.09
	B	1.52[b]		1		2.09
		0.35				0.89
Type IV						
Mean	A	2.22		1		2.27
SEM		0.60				1.33
	B	1.58[b]		1		2.07
		0.42				1.10

[a] D = deoxycholic acid.
[b] Significantly different from corresponding value on basal diet, $P < 0.05$.
[c] Significantly different from corresponding value on basal diet, $P < 0.01$.

The mechanisms regulating the transformation of cholesterol to C and CD are only known in part. Studies in the rat suggested the existence of compartmentation in the liver cholesterol pool, from which C, to a higher extent than CD, originates from newly synthesized material (1). The response to cholesterol feeding differs among species, the rats being characterized both by diminished cholesterogenesis and by enhanced bile acid formation. However, as shown in a recent report (31), the production of CD increases more than that of C. In keeping with this finding, the current data indicate that the normal human liver reacts to an increased dietary intake of cholesterol by a reduced production of C and/or a slightly augmented formation of CD. The absence of similar findings in the hypertriglyceridemic patients might be a further indication of abnormal metabolism of bile acids, cholesterol, or lipoproteins in these disorders.

A consistent finding in our previous studies of patients with the type II pattern was a subnormal C/CD ratio of the bile acids synthesized. The rapid normalization of the bile acid pattern upon administration of cholestyramine (3) demonstrated that the low formation of C under basal conditions may not be explained in terms of an inability to produce C. It was recently

TABLE 4. Biliary lipids when the subjects were fed basal (A) and cholesterol-rich (B) diets

Subject	Type of HLP[a]	Type of Diet	Cholesterol	Bile Acids	Phospholipids	Cholesterol Saturation[b]
				molar %		%
1.	N	A	5.5	85.0	9.6	121
		B	4.6	84.9	10.5	102
2.	N	A	4.6	82.9	12.5	99
		B	4.3	78.2	17.5	72
3.	N	A	5.6	79.7	14.8	99
		B	6.7	73.2	20.2	98
4.	N	A	9.8	74.1	16.1	163
		B	9.0	71.7	19.3	133
5.	N	A	5.2	73.7	21.1	76
		B	7.0	76.0	17.0	118
6.	N	A	6.8	74.7	18.5	105
		B	4.1	83.7	12.3	85
7.	IIb	A	12.1	71.6	16.3	196
		B	10.0	68.1	21.9	149
8.	IV	A	7.3	66.5	26.3	—
		B	6.6	62.7	30.8	
9.	IV	A	11.1	78.0	10.9	—
		B	10.4	66.5	23.1	
10.	IV	A	8.5	65.0	26.6	107
		B	8.6	68.2	23.3	117
11.	IV	A	13.0	65.4	21.6	181
		B	8.1	74.8	17.1	132
12.	IV	A	10.8	66.5	22.7	145
		B	13.1	58.4	28.5	179
Normal Mean ± SEM		A	6.3 ± 0.77	78.4 ± 2.00	15.4 ± 1.68	111 ± 12
		B	6.0 ± 0.80	78.0 ± 2.21	16.1 ± 1.59	101 ± 9
Type IIb + IV Mean ± SEM		A	10.5 ± 0.89[d]	68.8 ± 2.07[d]	20.7 ± 2.49	157 ± 20
		B	9.5 ± 0.91[c]	66.5 ± 2.27[d]	24.1 ± 2.00[c]	144 ± 13[c]
Type IV Mean ± SEM		A	10.1 ± 1.01[c]	68.3 ± 2.45[c]	21.6 ± 2.85	144 ± 21
		B	9.4 ± 1.11[c]	66.1 ± 2.75[d]	24.6 ± 2.38[c]	143 ± 18

[a] Abbreviations: HLP, hyperlipoproteinemia; N, normal.
[b] Calculated according to Carey and Small (15, 16) using a biliary lipid concentration of 10 g/dl.
[c] Significantly different from corresponding value in normolipidemic subjects $0.01 < P < 0.05$.
[d] Significantly different from corresponding value in normolipidemic subjects $0.001 < P < 0.01$.

reported that LDL rather than HDL transport cholesteryl esters to the splanchnic bed (32). Considering these and the current data, it is speculated that the high concentration of LDL cholesterol in HLP type II continuously stimulates the feed-back inhibition of hepatic cholesterogenesis. If so, a supranormal part of the body cholesterol in HLP type II might originate from extrahepatic sources, as the total cholesterol biosynthesis remains within normal limits (18).

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