Abnormal bile acid absorption in familial hypertriglyceridemia

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Abstract To better define the abnormality of bile acid metabolism associated with hypertriglyceridemia, we measured bile acid kinetics and absorption as well as preferential use of newly synthesized cholesterol for bile acid synthesis in eleven controls and ten subjects with hypertriglyceridemia, six of whom could be classified as having familial hypertriglyceridemia (FHT). Fractional turnover rates of cholic acid and chenodeoxycholic acid were both significantly elevated in hypertriglyceridemic subjects to nearly twice the rates in controls. Bile acid synthesis was also significantly higher in hypertriglyceridemic subjects while bile acid pools were either unchanged or somewhat reduced. Consistent with these kinetics, bile acid absorption was significantly lower in hypertriglyceridemic subjects than in controls. Overall only 10–12% of bile acid was derived from newly synthesized cholesterol, and hypertriglyceridemic subjects did not differ from controls. Because hypertriglyceridemia should not alter bile acid absorption, these results are consistent with the previously suggested possibility (B. Angelin, K. S. Hershon, and J. D. Brunzell. 1987. *Proc. Natl. Acad. Sci. USA.* **84:** 5434–5438) that impaired bile acid absorption may be a primary defect in some patients with hypertriglyceridemia.—**Duane, W. C.** Abnormal bile acid absorption in familial hypertriglyceridemia. *J. Lipid Res.* 1995. **36:** 96–107.

Supplementary key words triglyceride • cholesterol • hyperlipoproteinemia Type IV • cholelithiasis • atherosclerosis

A relationship between serum triglycerides and bile acid metabolism has been recognized for many years. Interruption of the enterohepatic circulation of bile acid increases serum triglyceride by increasing production of very low density lipoproteins (VLDL) (1–3). Partial suppression of bile acid synthesis by administration of chenodeoxycholic acid has the opposite effect (4, 5).

Conversely, a number of studies have shown that patients with elevated VLDL triglyceride have higher-than-normal rates of bile acid synthesis (6–9). In some of these studies, enhanced synthesis has been associated with increased size of bile acid pools (7, 8) and with increased rates of cholesterol synthesis (9, 10). A hypothesis suggested by these findings is that increased cholesterol synthesis is a primary abnormality which, in turn, results in enhanced bile acid synthesis.

More recently, Angelin, Hershon, and Brunzell (6) reported that the most pronounced abnormality of bile acid metabolism was in patients with familial hypertriglyceridemia (FHT) as opposed to patients with familial combined hyperlipidemia (FCHL), a classification scheme that post-dates many earlier studies of bile acid metabolism (11, 12). In contrast to earlier studies, elevated bile acid synthesis in these FHT patients was associated with increased fractional turnover of bile acid and unchanged bile acid pool size, suggesting that the increased synthesis might be secondary to reduced feedback inhibition. Increased fractional turnover of bile acid can result from either impaired bile acid absorption or more frequent cycling of the bile acid pool in the enterohepatic circulation (13). Although neither of these were directly measured by Angelin et al. (6), they did measure serum bile acids after a test meal and found significantly lower levels in FHT subjects. While this finding suggested some impairment of bile acid absorption in FHT, postprandial serum bile acid levels are a function of many variables including gastric emptying, gallbladder response to dietary components, small intestinal transit time, and hepatic extraction efficiency. Thus, from the observations available to date, we do not know whether high fractional turnover of bile acid in FHT patients is a result of increased enterohepatic cycling or impaired bile acid absorption. Indeed, we do not even know whether abnormal fractional turnover of bile acid in FHT can be confirmed.

One final possibility is that part of the abnormality of bile acid metabolism in hypertriglyceridemia may be a consequence of preferential use of newly synthesized cholesterol for bile acid synthesis, as has been hypothesized for the hypertriglyceridemia associated with the apolipoprotein E-2/2 phenotype (14). Studies in animal models (15, 16) have demonstrated that as much as 50%

Abbreviations: VLDL, very low density lipoprotein; FHT, familial hypertriglyceridemia; FCHL, familial combined hyperlipidemia.

of bile acid synthesis can preferentially derive from newly synthesized cholesterol (as opposed to cholesterol originating in equilibrated body pools), and that preferential use of newly synthesized cholesterol may contribute to regulation of bile acid synthesis (17). Studies in humans also suggest preferential use of newly synthesized cholesterol for bile acid synthesis (18, 19). However, there are no data bearing on the relationship of hypertriglyceridemia to preferential use of newly synthesized cholesterol for bile acid synthesis.

In the present study, we sought to better distinguish among these potential abnormalities, and to better characterize bile acid metabolism in patients with hypertriglyceridemia, especially those with FHT.

METHODS

We studied 21 male subjects, 11 with normal serum lipids and 10 with hypertriglyceridemia defined as a mean fasting triglyceride level exceeding 200 mg/dl. Detailed characterization of the subjects is provided in **Table 1.** Some of the data on subjects 1–6, 20, and 21 are also included in a study of effects of lovastatin (20). All subjects gave written informed consent to participate. Study procedures were approved by committees overseeing use of human subjects in research at both the Minneapolis VA Medical Center and the University of Minnesota.

Serum lipids including cholesterol, triglyceride, and HDL cholesterol were measured for each subject on at least three occasions by the clinical laboratory of the Minneapolis Veterans Affairs Medical Center. In a separate fresh serum sample, we separated the VLDL component by ultracentrifugation for measurement of triglycerides by a colorimetric method (21) and apolipoprotein B by a turbidimetric determination using a kit supplied by Boehringer Mannheim Biochemica (Indianapolis, IN). For the hypertriglyceridemic subjects, we contacted as many first degree relatives as possible. All relatives were asked to provide a small sample of venous blood for measurement of cholesterol, triglycerides, and HDL cholesterol. FHT is a subclassification of type IV hyperlipidemia characterized by elevated serum triglycerides with normal serum cholesterol, a high ratio of

TABLE 1. Subject characteristics

	Wt	Ht	BMI	Age	GS	Type	CH	TG	HDL	LDL
	kg	*m*	*kg/m²*	*yr*					*mg/dl*	
1	93.2	1.73	31.3	71	No	Nl	216	168	37	146
2	130.0	1.78	41.0	62	No	Nl	193	83	44	132
3	96.5	1.80	29.8	46	No	Nl	189	105	37	131
4	101.2	1.80	31.2	60	No	Nl	198	91	36	144
5	89.8	1.76	28.9	67	No	Nl	142	87	38	87
6	91.3	1.74	30.2	47	No	Nl	200	148	33	137
7	74.5	1.71	25.6	69	Yes	Nl	166	114	39	109
8	84.5	1.73	28.4	73	No	Nl	207	136	34	138
9	62.7	1.62	23.9	67	Yes	Nl	189	87	59	110
10	84.5	1.76	27.2	37	No	Nl	161	132	42	103
11	82.7	1.70	28.6	70	No	Nl	216	116	36	126
Normolipidemic subjects										
Mean	90.1	1.73	29.8	61			189	115	39	124
± SEM	5.1	0.02	1.3	4			7	8	2	6
12	107.7	1.65	39.6	61	No	FHT	207	238	33	133
13	80.5	1.59	31.9	60	Yes	FHT	136	201	28	79
14	115.5	1.75	37.7	62	No	FHT	230	385	44	123
15	123.6	1.84	36.6	59	No	FHT	203	261	30	141
16	102.7	1.76	33.1	61	No	FHT	214	209	37	152
17	110.5	1.74	36.6	68	No	FHT	174	262	30	97
18	95.5	1.71	32.5	55	No	?	183	326	24	85
19	95.9	1.73	32.2	68	No	?	193	292	28	118
20	75.7	1.64	28.2	74	No	?	193	361	27	40
21	81.6	1.69	28.6	49	No	?	233	370	27	132
All hypertriglyceridemic subjects										
Mean	98.9	1.71	33.6	62			197	291	31	110
± SEM	5.1	0.02	1.2	2			9	21	2	11
FHT subjects										
Mean	106.7	1.73	35.7	62			194	260	34	121
± SEM	6.0	0.04	1.2	1			14	27	2	11

Wt, weight; Ht, height; BMI, body mass index; GS, presence of gallstones; CH, cholesterol; TG, triglyceride; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; FHT, familial hypertriglyceridemic; Nl, normolipidemic.

triglyceride/apolipoprotein B in VLDL, and the absence of other types of hyperlipidemia in first degree relatives (6, 11, 12). For this study we selected only hypertriglyceridemic subjects with normal serum cholesterol. However, subjects were classified as having familial hypertriglyceridemia (FHT) only when they satisfied two additional criteria: *a)* at least three relatives were tested and all those with hypertriglyceridemia had normal levels of cholesterol; and *b)* the ratio of triglyceride/apolipoprotein B in VLDL exceeded 13.5, which is more than two standard deviations higher than the normal mean of 9.6 in our laboratory. If either criteria was not satisfied, the subject was classified as type unknown.

All subjects underwent evaluation of bile acid kinetics and biliary lipid metabolism as previously described (22). Briefly, this entailed administration of about 5 μCi each of [24-^{14}C]cholic acid and [24-^{14}C]chenodeoxycholic acid (New England Nuclear, Boston, MA) via duodenal tube followed by sampling of gallbladder bile on the 4 subsequent days. Each bile sample was analyzed for cholesterol, phospholipid, total bile acid, bile acid composition, bilirubin, and specific activity of cholic acid and chenodeoxycholic acid as described in previous publications (22–25). Also, on at least two separate occasions subjects underwent measurement of output of carbon monoxide on breath to estimate bilirubin production as previously described (25). These measurements permitted calculation of bile acid kinetics by the method of Lindstedt (26), cholesterol saturation index by the equations of Carey and Small (27), and secretion of lipids into bile by a method we have recently described (25). This latter method utilizes the fact that CO production rate reflects both bilirubin production and secretion. Biliary secretion for any bile constituent/bilirubin measured in gallbladder bile multiplied by the endogenous CO production rate. We have shown that this method accurately reflects cholesterol secretion compared to standard marker perfusion techniques, and that it is also more reproducible than marker perfusion (25). We used this method in part because of this reproducibility and in part because it is much easier for the study subjects than marker perfusion measurements. The method consistently provides an estimate of bile acid and lecithin secretion that is about 25% lower than those measured by marker perfusion. However, as all subjects in the present study were studied by the same method, the comparison of controls and hypertriglyceridemic subjects should be valid and informative.

Also, from these measurements we calculated fractional absorption of bile acid by the formula: $1 - [S/(O \times 24)]$ where S = daily synthesis of bile acid and O = hourly output of bile acid into bile. This calculation is based on the physiologic equivalence of bile acid synthesis and loss of bile acid in the fecal stream. Our own direct comparisons have confirmed this relationship, although in our laboratory measurements of bile acid synthesis consistently tend to be slightly (~15%) lower than measurements of fecal acidic sterol output (23). In addition, we calculated frequency of cycling of the bile acid pool in the enterohepatic circulation by the formula $(O \times 24)/P$ where P = total bile acid pool size. It should be noted that because our method of measuring bile acid secretion yields a consistently lower value than marker perfusion, the above estimates of both absorption and cycling will be somewhat lower than published by others. Again, however, because the same method was used to compare controls and hypertriglyceridemics, the comparison should provide valid estimates of relative differences.

Lastly five normal subjects (numbers 7–11) and eight hypertriglyceridemic subjects (numbers 12–19) underwent studies to determine the fraction of bile acid synthesis preferentially derived from newly synthesized cholesterol. For this purpose subjects were injected intravenously with 25–30 μCi of [4-^{14}C]cholesterol (New England Nuclear, Boston, MA) suspended in a fresh aliquot of the subject's own serum under aseptic conditions. Serum samples were drawn on or about days 1, 3, 5, 7, 10, 14, and weekly thereafter for 10–12 weeks. Aliquots of each serum sample were assayed for radioactivity and cholesterol mass by enzymatic assay for calculation of specific radioactivity. Time-dependence of specific activity was fitted to biexponential curves using the NLIN procedure of SAS (SAS Institute, Cary, NC) on a Northgate personal computer equipped with a 486DX microprocessor. On two separate occasions between weeks 4 and 7, each subject provided a sample of gallbladder bile as well as a serum sample. Bile samples were assayed for specific activity of cholic and chenodeoxycholic acid (22). The concomitant serum samples were analyzed for specific activity of free cholesterol using thin-layer chromatography and gas-liquid chromatography as previously described (28). The resulting values were used to calculate the fraction of bile acid synthesis derived preferentially from newly synthesized cholesterol as detailed in the Appendix.

Statistical testing was by unpaired *t*-test performed using SAS.

RESULTS

Fractional turnover rates of both cholic acid and chenodeoxycholic acid were significantly higher in the group of all hypertriglyceridemic subjects and in the subjects with FHT than in controls (**Table 2, Fig. 1**).

Total bile acid synthesis rate and synthesis rate of cholic acid were both significantly higher than control for the group of all hypertriglyceridemic subjects and the group of FHT subjects (Table 2). Synthesis rate for chenodeoxycholic acid showed the same trend but the changes were of borderline statistical significance (Table 2).

Mean pool sizes for cholic acid, chenodeoxycholic acid, and total bile acid all were smaller than control in hyper-

TABLE 2. Bile acid fractional turnover and synthesis

Subject	Fractional Turnover		Synthesis		
	CA	CDCA	CA	CDCA	Total
	1/day		*μmol/day*		
1	0.218	0.286	475	657	1132
2	0.240	0.070	590	231	821
3	0.366	0.292	978	593	1571
4	0.399	0.326	932	512	1444
5	0.797	0.558	1359	756	2120
6	0.419	0.189	637	541	1178
7	0.682	0.278	1499	888	2390
8	1.091	0.549	1352	810	2162
9	0.218	0.175	769	373	1142
10	0.272	0.245	1044	957	2000
11	0.238	0.162	802	424	1226
Normolipidemic subjects					
Mean	0.449	0.284	949	613	1560
± SEM	0.087	0.046	102	68	157
12	0.665	0.485	1127	761	1889
13	0.719	0.346	1757	713	2470
14	0.542	0.358	1447	727	2170
15	0.655	0.397	1603	1065	2670
16	1.136	0.760	1312	619	1930
17	0.812	0.474	1619	844	2460
18	1.325	0.913	2250	1607	3854
19	0.445	0.326	1200	868	2070
20	0.449	0.286	755	557	1312
21	0.599	0.479	814	637	1451
All hypertriglyceridemic subjects					
Mean	0.735	0.482	1388	840	2230
± SEM	0.091	0.064	142	97	227
P-value	0.036	0.019	0.020	0.066	0.024
FHT subjects					
Mean	0.755	0.470	1478	788	2266
± SEM	0.084	0.063	94	63	130
P-value	0.037	0.030	0.004	0.112	0.009

FHT, familial hypertriglyceridemic; CA, cholic acid; CDCA, chenodeoxycholic acid.

triglyceridemic subjects, but none of the comparisons reached statistical significance (**Table 3**). Proportion of bile acids in the pool was not statistically significant for hypertriglyceridemic subjects versus controls, although percentage of the pool represented by deoxycholic acid tended to be higher in hypertriglyceridemic subjects (**Table 4**).

Calculated rates of bile acid absorption were significantly reduced in the group of all hypertriglyceridemic subjects and in the FHT subjects compared to control (Table 3, **Fig. 2**). Frequency of cycling of the bile acid pool in the enterohepatic circulation was not different in hypertriglyceridemic subjects compared to controls (Table 3).

Secretion of neither bile acid nor lecithin into bile was significantly different in hypertriglyceridemic subjects compared to control, although mean bile acid secretion was lower in both groups of hypertriglyceridemic subjects than in the control group (**Table 5**). Secretion of cholesterol into bile was significantly higher in both groups of hypertriglyceridemic subjects than in controls (Table 5).

Cholesterol saturation index of gallbladder was also significantly higher in the group of all hypertriglyceridemic subjects and the FHT subjects compared to controls (**Table 6**). Molar percent of bile acid was significantly lower in both groups with hypertriglyceridemia compared to controls while molar percents of lecithin and cholesterol were significantly higher in hypertriglyceridemia than in controls (Table 6).

Measured and expected specific activities of bile acid relative to free cholesterol in serum along with calculated percentages of bile acid synthesis preferentially derived from newly synthesized cholesterol are presented in **Table 7.** On average, only 10–12% of bile acid synthesis was derived from newly synthesized cholesterol. Mean percentages for cholic acid were similar to those for chenodeoxycholic acid. There were no significant differences in use of newly synthesized cholesterol for bile acid synthesis for hypertriglyceridemic subjects versus controls.

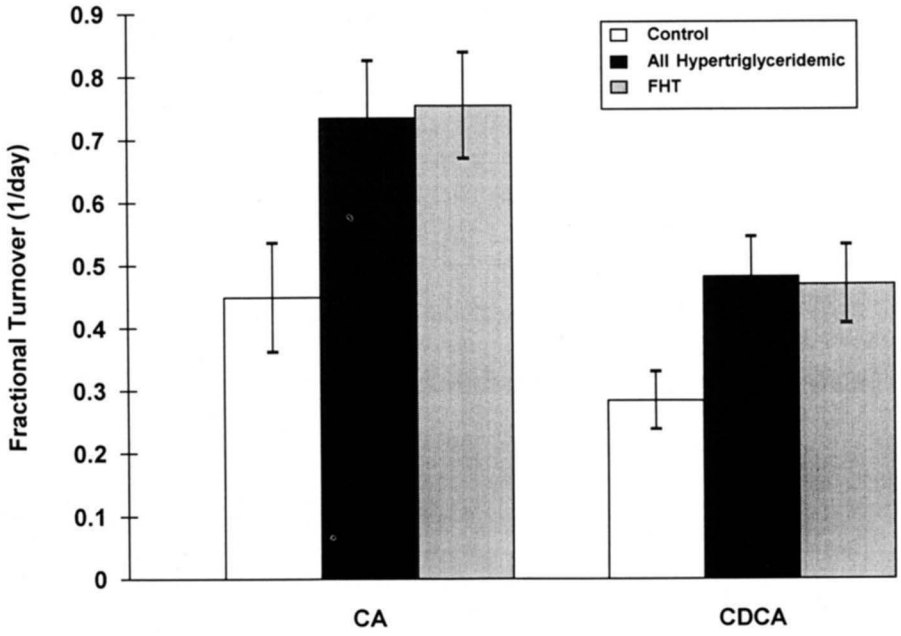

Fig. 1. Fractional turnover rates for cholic acid (CA) and chenodeoxycholic acid (CDCA) in controls, all hypertriglyceridemic subjects, and subjects classified as having FHT. Both groups of hypertriglyceridemic subjects had rates significantly higher than control for both bile acids.

Cholesterol production rate, calculated by standard formulas from the serum cholesterol specific activity-time relation (29), averaged 3.43 mmol/day in controls, 5.52 mmol/day in the combined group of hypertriglyceridemic patients ($P = 0.0008$), and 5.68 mmol/day in the group of FHT subjects ($P = 0.0017$). There was no relationship between the percent of bile acid derived from newly synthesized cholesterol and either cholesterol production ($r = 0.15$, $P = 0.631$) or bile acid synthesis ($r = 0.14$, $P = 0.642$).

We also re-analyzed the data excluding the three subjects with gallstones (Table 1). No comparison between hypertriglyceridemic subjects and controls that was not significant before ($P < 0.05$) became significant after this exclusion. With only one exception, all comparisons of hypertriglyceridemic subjects to controls that were significant before remained significant after exclusion of gallstone subjects. The exception was secretion of cholesterol into bile ($P = 0.049$ before exclusion, $P = 0.165$ after exclusion).

To assess the need for correcting certain parameters for body weight, body mass index, or age, we performed the regression analysis summarized in **Table 8.** No parameter of bile acid metabolism approached a statistically significant correlation with any of these three variables. This suggested that age and obesity, at least in this group of subjects, had no important effect on bile acid metabolism, and that correction for weight and/or age would be inappropriate and perhaps misleading. Correlation of cholesterol production with weight and body mass index was significant, as would be expected from known effects of obesity of cholesterol synthesis (30–32). We did not correct this parameter because its magnitude was not an integral result of the study. Correlation of cholesterol secretion and saturation index with both body weight and body mass index approached, but did not reach, statistical significance, perhaps reflecting, in part, the known influence of cholesterol synthesis on biliary cholesterol secretion (33).

DISCUSSION

It is usually desirable to study as homogeneous a group of subjects as possible, but in the present study, as in most demanding human studies, that goal was not perfectly met. Thus not all our hypertriglyceridemic subjects could be unequivocally classified as having FHT. On the other hand, the statistical comparisons and conclusions were not appreciably altered when we compared controls to only those classified as FHT versus the entire group of hypertriglyceridemics. This may have been because some of our unclassified subjects, all of whom had normal serum cholesterol levels and some of whom were classified as unknown because of unavailability of family members, actually had FHT. Thus none of our results should be

TABLE 3. Bile acid pools, absorption, and enterohepatic cycling

Subject	Pools			Absorption	Cycling
	CA	CDCA	Total		
		μmol		*fraction*	*1/day*
1	2180	2290	6060	0.971	6.36
2	2460	3320	8270	0.977	4.29
3	2670	2030	7510	0.942	3.60
4	2340	1570	8190	0.941	2.98
5	1705	1353	4000	0.890	4.82
6	1520	2870	4900	0.952	5.03
7	2200	3200	9280	0.851	1.72
8	1239	1476	4110	0.884	4.53
9	3530	2140	6750	0.954	3.65
10	3840	3910	10040	0.919	2.45
11	3360	2610	8020	0.955	3.37
Normolipidemic subjects					
Mean	2460	2430	7010	0.930	3.89
± SEM	253	250	614	0.012	0.39
12	1696	1569	4350	0.900	4.36
13	2440	2060	8620	0.934	4.31
14	2670	2030	6580	0.864	2.43
15	2450	2680	8680	0.913	3.52
16	1155	815	3990	0.874	3.86
17	1995	1780	5620	0.816	2.38
18	1696	1760	4550	0.846	5.51
19	2700	2670	7120	0.887	2.58
20	1680	1945	5780	0.935	3.50
21	1360	1331	4710	0.951	6.34
All hypertriglyceridemic subjects					
Mean	1980	1860	6000	0.892	3.88
± SEM	175	179	541	0.014	0.41
P-value	0.146	0.085	0.235	0.046	0.984
FHT subjects					
Mean	2070	1820	6310	0.884	3.48
± SEM	233	253	832	0.017	0.36
P-value	0.327	0.137	0.505	0.037	0.499

FHT, familial hypertriglyceridemic; CA, cholic acid; CDCA, chenodeoxycholic acid.

taken as applicable to any group of hypertriglyceridemic subjects other than FHT. It is also worth noting that separation of subjects into FHT versus FCHL is often problematical and may to some extent be artificial. Nevertheless, the criteria embodied in the FHT classification do seem to define a subset of hypertriglyceridemic subjects with distinctive abnormalities of bile acid metabolism as delineated in the present study and that of Angelin et al. (6).

Finally, an additional nonhomogeneity in the study group was the presence of gallstones in three subjects (Table 1). However, excluding these three subjects from the statistical analysis did not materially alter the results (see Results).

We also attempted to select a control group that would, as much as possible, match the hypertriglyceridemic subjects. The match for sex and age was excellent (Table 1). The hypertriglyceridemic subjects had a somewhat larger mean body mass index than the controls (Table 1), again reflecting the difficulty in recruiting healthy subjects for such arduous studies. However, the difference was relatively small, and there was no correlation between body mass index versus any parameter of bile acid metabolism measured (Table 8). It is unlikely, therefore, that this marginal difference in body mass index accounts for the rather large differences observed between controls and hypertriglyceridemic subjects for bile acid turnover, synthesis, and absorption.

A critical finding in the present study was that hypertriglyceridemic subjects, and more specifically FHT subjects, had much higher fractional turnover of bile acid than normolipidemic controls (Table 2, Fig. 1). Bile acid synthesis was also higher in hypertriglyceridemic subjects than controls (Table 2). While several previous studies have demonstrated high rates of bile acid synthesis in hypertriglyceridemia (6-9), only recently has a study shown increased fractional turnover of bile acid (6), perhaps because that study focused on FHT subjects.

TABLE 4. Proportions of bile acid in gallbladder bile

Subject	LCA	DCA	CDCA	UDCA	CA
1	1.0	17.8	48.2	3.3	29.6
2	1.9	25.8	34.4	2.2	35.7
3	2.5	31.3	30.9	3.7	31.6
4	1.8	47.4	20.1	3.4	27.3
5	3.5	19.6	37.6	0.6	38.7
6	2.7	6.1	55.1	3.0	33.1
7	2.1	32.6	37.4	6.0	22.0
8	1.3	29.8	37.6	2.5	28.9
9	1.2	13.3	32.7	2.1	50.7
10	2.5	16.3	42.5	3.3	35.3
11	2.3	22.4	35.4	1.2	38.7
Normolipidemic subjects					
Mean	2.1	23.9	37.4	2.8	33.8
± SEM	0.2	3.4	2.8	0.4	2.3
12	2.4	21.8	37.0	0.7	38.1
13	2.3	41.4	29.0	3.3	24.1
14	1.3	24.0	33.4	3.6	37.7
15	3.3	35.7	34.9	0.8	25.3
16	2.1	44.7	21.2	4.0	27.9
17	2.8	27.1	34.9	2.7	32.5
18	1.5	19.1	40.6	3.1	35.6
19	3.7	17.1	38.0	3.8	37.4
20	1.7	32.6	32.7	3.0	30.0
21	2.2	37.4	30.6	3.0	26.8
All hypertriglyceridemic subjects					
Mean	2.3	30.1	33.3	2.8	31.5
± SEM	0.2	3.0	1.7	0.4	1.7
P-value	0.443	0.191	0.220	0.937	0.448
FHT subjects					
Mean	2.4	32.5	31.7	2.5	30.9
± SEM	0.3	3.9	2.4	0.6	2.5
P-value	0.432	0.135	0.189	0.656	0.441

FHT, familial hypertriglyceridemic; LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid.

Although postprandial serum bile acids were abnormally low in those FHT patients, so many variables affect serum bile acid levels that this finding does not permit any conclusion about the mechanism responsible for the observed increase in fractional turnover.

Fractional turnover of a bile acid pool is mathematically identical to the product of enterohepatic cycling frequency and the fraction of bile acid lost during passage through the intestine. Fraction of bile acid lost per cycle is determined mainly by absorption, although for primary bile acids, conversion to secondary bile acids may also contribute. Cycling frequency depends on rates of individual transport processes in the enterohepatic cycle, but is largely determined by the slowest components such as small intestinal transit and gallbladder emptying (22, 34). Because none of these processes would be expected to depend on bile acid synthesis, it is quite unlikely that the increased bile acid synthesis in our hypertriglyceridemic subjects caused the observed high fractional turnover.

On the other hand, some causes of increased fractional turnover of bile acid can result in increased bile acid synthesis. Because bile acid synthesis is under negative feedback control, decreasing absorption of bile acid, as with cholestyramine administration (1–3), will predictably increase synthesis rate. Theoretically, increased conversion of primary to secondary bile acid could similarly increase synthesis depending on the fraction of the secondary bile acid reabsorbed and inhibitory potency of the secondary versus the primary bile acid. In contrast, increasing the rate of enterohepatic cycling would not be expected to increase synthesis. Such a change would temporarily increase hepatic flux of bile acid causing a decrease in syn-

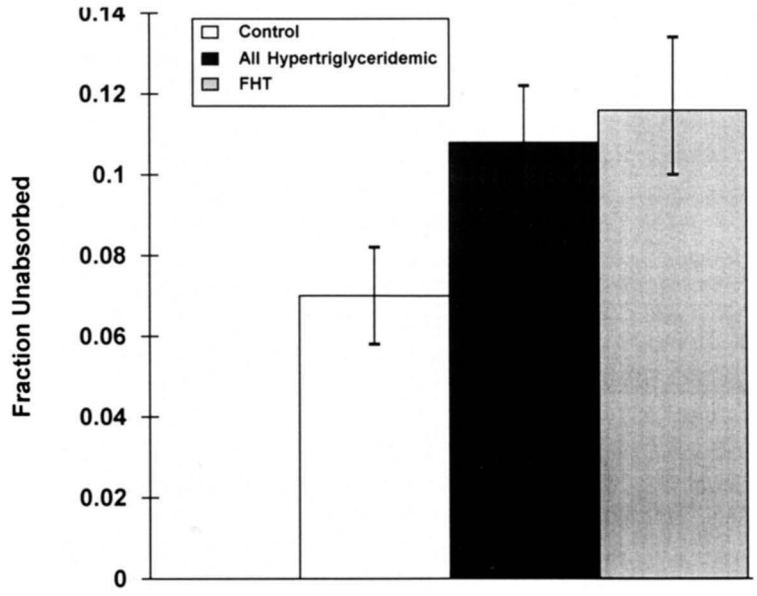

Fig. 2. Calculated fraction of bile acid unabsorbed in controls, all hypertriglyceridemic subjects, and FHT subjects. Both groups of hypertriglyceridemic subjects appeared to absorb a significantly lower fraction of bile acid than controls.

TABLE 5. Biliary lipid secretion rates

Subject	Bile Acid	Lecithin	Cholesterol
		μmol/h	
1	1605	381	139
2	1479	473	132
3	1127	292	84
4	1017	337	172
5	803	152	60
6	1026	258	111
7	666	209	57
8	775	185	69
9	1028	169	55
10	1027	290	67
11	1126	446	104
Normolipidemic subjects			
Mean	1062	290	96
± SEM	85	33	12
12	790	252	107
13	1550	465	185
14	667	300	113
15	1274	468	202
16	640	257	160
17	557	176	84
18	1044	299	113
19	764	271	108
20	842	256	130
21	1245	351	157
All hypertriglyceridemic subjects			
Mean	937	310	136
± SEM	103	30	12
P-value	0.360	0.667	0.030
FHT subjects			
Mean	913	320	142
± SEM	165	49	19
P-value	0.384	0.613	0.049

FHT, familial hypertriglyceridemic.

thesis. The pool would then shrink to the point at which hepatic flux and synthesis had returned to control levels. Thus the combination of increased bile acid synthesis, increased fractional turnover, and normal pool sizes seen in our group of hypertriglyceridemic subjects is theoretically consistent with impaired bile acid absorption or possibly increased conversion of primary to secondary bile acid, but would not be well explained by increased enterohepatic cycling or a primary increase in bile acid synthesis.

The above considerations are based solely on the bile acid kinetic data, as presented in both the present study and the paper of Angelin et al. (6). We also went a step further by measuring hepatic secretion of bile acid (Table 5), providing a quantitative estimate for flux of bile acid in the enterohepatic circulation. Calculations based on these measurements confirmed our conclusions from the kinetic data alone, namely that the hypertriglyceridemic subjects had impaired bile acid absorption with a normal rate of enterohepatic cycling (Table 3, Fig. 2). From two approaches, then, our findings support the hypothesis that increased fractional turnover in hypertriglyceridemia results, at least in part, from impaired bile acid absorption.

We did not assess rate of conversion of primary to secondary bile acid in our subjects. In light of the observed reduction in bile acid absorption, such an increased conversion to secondary bile acids is unlikely to be the sole cause of increased fractional turnover in hypertriglyceridemia. In fact, Carella, Einarsson, and Hellström (35) have reported that patients with Type IV hyperlipidemia have normal rates of deoxycholic acid production. Conceivably, however, increased conversion to secondary bile acids could have been present, as suggested by the tendency toward a high proportion of deoxycholic acid in the bile of hypertriglyceridemic subjects (Table 4). In fact, one might expect such increased conversion to occur as a result of decreased absorption of primary bile acid. Whether or not increased conversion to secondary bile acids might be present as an additional de novo abnormality in hypertriglyceridemia cannot be ascertained from the present study and indeed might be very difficult to ascertain at all.

The reason for impaired bile acid absorption in FHT patients is unknown. It is conceivable that a high level of triglyceride or some associated metabolic abnormality

TABLE 6. Gallbladder bile lipid composition and saturation index

Subject	Bile Acid	Lecithin	Cholesterol	CSI
		molar percent		
1	73.3	19.1	7.61	1.14
2	70.6	22.9	6.47	0.88
3	75.2	19.2	5.59	0.86
4	66.5	22.2	11.36	1.53
5	78.5	15.5	6.02	1.07
6	73.5	18.5	7.94	1.23
7	71.4	22.4	6.16	0.85
8	75.3	18.0	6.67	1.06
9	82.1	13.5	4.42	0.87
10	74.0	21.1	4.89	0.72
11	68.4	25.2	6.37	0.88
Normolipidemic subjects				
Mean	73.5	19.8	6.68	1.01
± SEM	1.3	1.0	0.56	0.07
12	68.8	21.8	9.36	1.30
13	66.1	24.1	9.78	1.33
14	61.4	28.0	10.68	1.31
15	64.8	24.4	10.85	1.40
16	60.4	24.4	15.24	1.93
17	67.4	22.2	10.48	1.45
18	71.9	20.4	7.69	1.12
19	66.7	23.8	9.50	1.25
20	67.3	21.7	10.96	1.54
21	70.9	20.1	9.02	1.31
All hypertriglyceridemic subjects				
Mean	66.6	23.1	10.36	1.39
± SEM	1.2	0.7	0.63	0.07
P-value	0.001	0.019	0.001	0.001
FHT subjects				
Mean	64.8	24.1	11.06	1.45
± SEM	1.4	0.9	0.87	0.10
P-value	0.001	0.013	0.001	0.002

FHT, familial hypertriglyceridemic; CSI, cholesterol saturation index.

TABLE 7. Bile acid derived from newly synthesized cholesterol

Subject	Specific Activity: Measured CA	Measured CDCA	Expected[a] CA	Expected[a] CDCA	Production from NSC CA	Production from NSC CDCA	Production from NSC Total
			percent free cholesterol			*percent synthesis*	
7	93.3	99.4	103.3	108.6	9.7	8.4	9.2
8	85.7	91.1	101.4	102.8	15.4	11.3	13.9
9	100.0	104.4	104.6	105.8	4.4	1.3	3.4
10	92.0	95.3	108.7	109.7	15.4	13.2	14.3
11	89.9	87.3	106.7	110.2	15.7	20.7	17.5
Normolipidemic subjects							
Mean	92.2	95.5	104.9	107.4	12.1	11.0	11.7
± SEM	2.3	3.0	1.3	1.4	2.2	3.2	2.4
12	94.5	103.4	104.3	106.0	9.4	2.4	6.6
13	97.7	112.2	104.9	110.8	11.0	9.3	10.5
14	89.1	96.5	104.8	107.4	14.9	10.2	13.3
15	78.6	87.8	101.9	103.1	22.9	14.8	19.7
16	93.5	85.5	101.7	102.6	8.0	16.6	10.8
17	88.1	95.7	102.6	104.6	14.2	8.5	12.2
18	98.4	97.8	101.0	101.5	2.6	3.6	3.0
19	101.4	107.1	106.8	109.6	5.1	2.3	3.9
All hypertriglyceridemic subjects							
Mean	92.7	98.3	103.5	105.7	11.0	8.5	10.0
± SEM	2.6	3.2	0.7	1.2	2.2	1.9	1.9
FHT subjects							
Mean	90.2	96.9	103.4	105.7	13.4	10.3	12.2
± SEM	2.7	4.0	0.6	1.2	2.2	2.1	1.8

FHT, familial hypertriglyceridemic; CA, cholic acid; CDCA, chenodeoxycholic acid; NSC, newly synthesized cholesterol.
[a]See Appendix for calculations.

might reduce bile acid absorption, although there is no known mechanism by which this could occur. Alternatively, the abnormal bile acid absorption in these patients might be a primary abnormality contributing to hypertriglyceridemia. That possibility is supported by the fact that artificially reducing bile acid absorption with cholestyramine can induce hypertriglyceridemia, although usually not to the degree found in FHT subjects (1-3). The present study is consistent with this second possibility, but falls far short of proving it.

TABLE 8. Correlation coefficients for weight, BMI, and age

Variable	Weight *r*-Value	Weight *P*-Value	BMI *r*-Value	BMI *P*-Value	Age *r*-Value	Age *P*-Value
CA fractional turnover	0.13	0.563	0.19	0.414	0.10	0.668
CDCA fractional turnover	0.12	0.612	0.15	0.525	−0.01	0.981
Bile acid absorption	−0.21	0.365	−0.24	0.304	−0.18	0.440
CA synthesis	0.20	0.374	0.26	0.256	0.01	0.962
CDCA synthesis	0.08	0.737	0.06	0.783	−0.17	0.458
Total synthesis	0.16	0.479	0.19	0.403	−0.06	0.786
CA pool	−0.12	0.605	−0.17	0.450	−0.17	0.452
CDCA pool	0.02	0.944	−0.07	0.771	−0.26	0.255
Total pool	0.02	0.918	−0.07	0.767	−0.19	0.397
Bile acid secretion	−0.06	0.811	−0.06	0.781	−0.22	0.334
Lecithin secretion	0.32	0.155	0.31	0.165	−0.17	0.467
Cholesterol secretion	0.36	0.104	0.37	0.094	−0.09	0.696
Cholesterol saturation index	0.28	0.210	0.35	0.125	0.18	0.446
Cholesterol production	0.67	0.011	0.69	0.009	−0.24	0.423

BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid.

An alternative hypothesis has been that increased cholesterol synthesis in hypertriglyceridemia is a primary abnormality leading to increased production of both VLDL and bile acid. A number of studies have documented increased bile acid synthesis in hypertriglyceridemia in association with increased cholesterol synthesis (7–10). Obesity is a common finding in association with increased synthesis of both cholesterol (30, 32) and bile acid (36), making cause-and-effect conclusions even more difficult to draw.

Although cholesterol production was increased in a subset of our hypertriglyceridemic subjects (Results), it is likely that this was in part a result of obesity because there was a significant correlation of cholesterol production with body mass index in that subset of subjects (Table 8). Even if we had found increased cholesterol production in the entire group unassociated with obesity, we would not have known to what extent it was a primary abnormality versus a compensatory response to impaired bile acid absorption as seen during cholestyramine treatment (3). Finally, as noted above, the hypothesis that increased cholesterol synthesis is driving increased bile acid synthesis does not explain the high fractional turnover of bile acid seen in hypertriglyceridemic subjects both in this study and that of Angelin et al. (6).

An additional potential component of this hypothesis is that patients with hypertriglyceridemia may have increased rates of bile acid synthesis perhaps because they derive a greater than normal fraction of bile acid synthesis from newly synthesized cholesterol (14), a factor thought to partially regulate bile acid synthesis in animal models (15–17). However, we found no difference between hypertriglyceridemic subjects and controls in this parameter. Indeed, on average only 10–12% of bile acid synthesis was derived from newly synthesized cholesterol (Table 7). Although an earlier study in bile fistula subjects suggested that perhaps 30% of bile acid synthesis arose from newly synthesized cholesterol in humans (19), a more recent study from the same laboratory found that the number was closer to 6% (18), similar to the mean of about 10–12% found in our subjects. It seems likely, therefore, that preferential use of newly synthesized cholesterol is not a regulatory factor in either normal or hypertriglyceridemic humans.

Hypertriglyceridemia is a known risk factor for cholelithiasis (37–39) and has been associated with supersaturated bile in some studies (40), although not in others (6). We found a significantly increased cholesterol saturation index in our hypertriglyceridemic subjects compared to controls. This resulted in part from a significantly increased rate of cholesterol secretion into bile (Tables 5 and 6), an abnormality that apparently has not been previously reported in hypertriglyceridemia. Because our hypertriglyceridemic subjects had a slightly higher body mass index compared to controls, it is possible that obesity contributed somewhat to this increased cholesterol secretion and saturation index. However, neither cholesterol secretion nor saturation index correlated significantly with body mass index (Table 8).

In addition to abnormalities in biliary cholesterol, molar percent bile acid was disproportionately lower and molar percent lecithin was significantly higher in hypertriglyceridemics compared to controls. This combination would not be expected when saturation index is high because of high cholesterol secretion alone (41) and suggests that impaired bile acid absorption may also have contributed to excess cholesterol saturation of gallbladder bile. Bile acid secretion and bile acid pool sizes were also lower in hypertriglyceridemic subjects, although neither change reached statistical significance. That neither cholestyramine administration nor ileal resection increased cholesterol saturation index (42–45) does not necessarily disprove this hypothesis. Ileal resection lowers absorption of cholesterol as well as bile acid (46). Cholestyramine binds a variety of substances, binds some bile acids better than others, impairs absorption throughout the length of small bowel rather than just at the terminal ileum, and may also reduce absorption of cholesterol, at least by some methods of measurement (46, 47). Thus, further study will be needed to fully understand the effects of impaired bile acid absorption on cholesterol saturation in FHT.

In conclusion, the present study provides direct evidence for impaired bile acid absorption in FHT. A logical next step would be to separately examine passive and active components of bile acid absorption in FHT patients. One possibility is that patients with FHT may have a primary genetic abnormality in bile acid transport. Angelin et al. (6) originally proposed this hypothesis and pointed out that such a primary abnormality in bile acid absorption could lead to increased production of both bile acid and VLDL. However, because stimulation of bile acid synthesis leads to relatively small increases in serum triglycerides (1–3), it seems unlikely that a primary defect in bile acid absorption could fully explain the level of serum triglycerides often seen in FHT.

APPENDIX

We labeled body cholesterol pools with [4-^{14}C]cholesterol to determine the fraction of bile acid synthesis arising preferentially from newly synthesized cholesterol. To a first approximation, this fraction can be estimated by direct comparison of the specific activity of free cholesterol to that of the two primary bile acids, cholic acid and chenodeoxycholic acid. However, that approach neglects the fact that there is a pool of bile acid with a turnover that is not instantaneous. Correction for bile acid turnover requires knowledge of turnover rates for both cholesterol and bile acid.

According to Goodman, Noble, and Dell (29), for the first 12 weeks after administration of isotopic cholesterol, the serum cholesterol specific activity decay curve is best fitted as a biexponential. (Continuation of measurements beyond 12 weeks requires fitting to a triexponential (29), but our experiments were not so prolonged.) Thus, the serum cholesterol

specific activity at any point in time (SA_C) is described by the following equation:

$$SA_C = Ae^{-\alpha t} + Be^{-\beta t} \quad Eq.\ 1)$$

The constants α and β are usually about 0.15 and 0.015 $days^{-1}$, respectively, and A and B are usually about 1700 and 550 dpm/mg, respectively (29). Using these approximate values it can be shown that as the time after isotope administration exceeds 4 weeks, the first exponential accounts for <7% of the total, and SA_C can be approximated as:

$$SA_CC = Be^{-\beta t} \quad Eq.\ 2)$$

We also assume the cholic acid pool (C) to be constant and to turnover with monoexponential kinetics. The rate constant for this process is k. Synthesis rate of cholic acid is also a constant represented by s, and:

$$C = s/k \quad Eq.\ 3)$$

The pool of [^{14}C]cholic acid at any time is defined as C*. It also turns over with monoexponential kinetics so that:

$$dC^*/dt = -kC^* + s^* \quad Eq.\ 4)$$

Where s* = rate of input of [^{14}C]cholic acid

Assuming the pool of cholesterol used for cholic acid synthesis is in perfect equilibrium with free cholesterol in the serum:

$$s^* = (s)\ (SA_C) = (s)\ (Be^{-\beta t}) \quad Eq.\ 5)$$

Then:

$$dC^*/dt = -kC^* + (s)\ (B)e^{-\beta t} \quad Eq.\ 6)$$

The solution to this differential equation is:

$$C^* = [(sB)/(k-\beta)]e^{-\beta t} \quad Eq.\ 7)$$

Then the cholic acid specific activity (SA_B) is:

$$SA_B = C^*/C = [(kB)/(K-\beta)]e^{-\beta t} \quad Eq.\ 8)$$

and rearranging:

$$SA_B = [k/(k-\beta)]SA_C \quad Eq.\ 9)$$

A completely analogous calculation can be made for chenodeoxycholic acid.

The rate constant k for both cholic and chenodeoxycholic acid was determined by standard Lindstedt kinetics (see Methods). The rate constant, β, was derived from curve fitting of specific activity decay curves following administration of [4-^{14}C]cholesterol. On two separate occasions at least 4 weeks after administration of [4-^{14}C]cholesterol, we simultaneously measured specific activities of serum free cholesterol and biliary cholic and chenodeoxycholic acids. The expected (assuming no preferential use of newly synthesized cholesterol for bile acid synthesis) values for specific activities of these two bile acids were calculated from equation 9 above. For both primary bile acids, the fraction of synthesis derived preferentially from newly synthesized cholesterol (F) was calculated as:

$$F = 1 - [SA_{B(ms)}/SA_{B(ex)}] \quad Eq.\ 10)$$

Where $SA_{B(ms)}$ is the measured specific activity of cholic or chenodeoxycholic acid and $SA_{B(ex)}$ is the expected specific activity of that same bile acid.

Finally, the fraction of total bile acid synthesis derived preferentially from newly synthesized cholesterol was calculated as the weighted average of F values for cholic and chenodeoxycholic acids weighted for the fraction each bile acid contributed to total bile acid synthesis (Table 2).

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