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Bile acid metabolism in hereditary forms of hypertriglyceridemia: Evidence for an increased synthesis rate in monogenic familial hypertriglyceridemia

(cholesterol/triglyceride/familial combined hyperlipidemia)

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ABSTRACT **This study was undertaken to characterize bile acid metabolism in hereditary forms of hypertriglyceridemia. Ten hypertriglyceridemic patients (type IV phenotype) with familial combined hyperlipidemia and 7 patients with monogenic familial hypertriglyceridemia (FHTG) were compared with 18 healthy controls; all subjects were males. Pool size, synthesis rate, and fractional catabolic rate of cholic and chenodeoxycholic acids were determined with an isotope dilution technique. Patients with FHTG had synthesis rates of cholic acid, chenodeoxycholic acid, and total bile acids above those seen in normal controls ($P < 0.001$); also the fractional catabolic rates of both bile acids were increased ($P < 0.001$). In contrast, bile acid kinetic parameters were—with one exception—within normal limits in patients with familial combined hyperlipidemia. The abnormality of bile acid metabolism could also be identified in a normolipidemic individual presumed to carry the gene for FHTG. The postprandial rise of serum bile acids was blunted in FHTG, indicating that the intestinal uptake of bile acids may be deficient in this condition. We conclude that FHTG, but not familial combined hyperlipidemia, is frequently associated with a defective regulation of bile acid synthesis, resulting in an abnormally high production rate of bile acids. It is hypothesized that this abnormality is important for the subsequent development of hypertriglyceridemia.**

Two common inherited disorders of lipoprotein metabolism that are associated with increased levels of very low density lipoproteins (VLDL) have been described (1). One is familial combined hyperlipidemia (FCHL), characterized by the presence of multiple lipoprotein phenotypes (IIa, IIb, and IV) among the relatives of a family (2–4) and in a single individual studied on different occasions (5). The other is familial hypertriglyceridemia (FHTG), in which hypertriglyceridemia (phenotype IV) is found in all affected family members (1, 2).

Although FCHL and FHTG may be difficult to separate in some cases, numerous studies indicate that they are different metabolic entities. VLDL apolipoprotein (apo) B synthesis rates appears to be distinctly elevated in hypertriglyceridemic subjects (phenotype IIb or IV) with FCHL (6–8). Patients with FHTG, on the other hand, have normal (7, 8) or mildly elevated (6) VLDL apo-B synthesis rates that are lower than those seen in FCHL (6, 7). In contrast, plasma VLDL triglyceride synthesis rates are higher in patients with FHTG than in those with FCHL (6, 8). It is therefore assumed that the hypertriglyceridemia of FCHL is primarily due to overproduction of VLDL apo-B, whereas that seen in FHTG seems to be primarily due to overproduction of VLDL triglycerides (6). Furthermore, individuals with FCHL and FHTG appear to differ in apparent risk for developing coronary artery disease (9) and in the relationship between triglyceride levels and insulin and obesity (10, 11).

A common but not universal finding in patients with hypertriglyceridemia is overproduction of bile acids and cholesterol (for review, see ref. 12). Individuals with a high synthesis rate of bile acids also display an increased rate of VLDL triglyceride synthesis (13). Whether the enhanced bile acid production in some patients with type IV pattern is a primary or a secondary phenomenon cannot be decided presently. However, several lines of evidence indicate that the metabolism of VLDL triglycerides is influenced by disturbances of bile acid metabolism (12). Thus, induction of bile acid biosynthesis with cholestyramine treatment or biliary drainage is often linked to an increase in plasma triglyceride production rate (14, 15). On the other hand, suppression of bile acid biosynthesis by feeding with chenodeoxycholic acid (CDCA; 3α,7α-dihydroxy-5β-cholanic acid) is linked to a reduced synthesis of plasma VLDL triglyceride (14, 16).

The heterogeneity of patients with type IV phenotype in regard to bile acid synthesis rate led us to ask the questions: (*i*) Do FCHL and FHTG differ in the abnormality of bile acid metabolism frequently seen in type IV hyperlipoproteinemia? If so, (*ii*) could this disturbance have any importance in the development of hypertriglyceridemia? In this report, we present evidence that FHTG, but not FCHL, is associated with abnormal bile acid metabolism, and that this abnormality may be a primary defect in some patients with monogenic FHTG.

MATERIALS AND METHODS

Patients. Altogether, 17 male patients with hypertriglyceridemia were studied—ten in Stockholm and the remainder in Seattle (Table 1). Their ages ranged from 23 to 68 (mean age, 53 yr), and, with one exception, they were not obese. They were propositi or members of families previously characterized for genetic hyperlipoproteinemia. Laboratory and clinical investigations excluded the presence of secondary hyperlipoproteinemia and type III hyperlipoproteinemia, and a mean of seven first-degree relatives (range, 5–11) per index patient had been characterized. The criteria used to determine FCHL or FHTG were modifications (5, 9) of those previously reported (2). Patients were selected for metabolic studies only if they came from FHTG families in which all affected relatives were lipoprotein phenotype IV or from families with FCHL in which at least one affected relative had

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Abbreviations: apo, apolipoprotein; CA, cholic acid; CDCA, chenodeoxycholic acid; FCHL, familial combined hyperlipidemia; FHTG, familial hypertriglyceridemia; FCR, fractional catabolic rate; VLDL, very low density lipoprotein(s).

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Table 1. Patient characterization

Subject	Age, yr	Body wt, kg	Relative body wt, %	Cholesterol, mg/dl	Triglyceride, mg/dl	Range of lipid concentration, mg/dl	
						Cholesterol	Triglyceride
				Familial hypertriglyceridemia			
WM*	68	93	127	324	1420	202–324	390–1420
BJo	67	67	103	143	469	136–179	422– 610
TaE†	56	82	94	194	334	178–209	309– 372
TuE†	56	83	93	232	496	213–252	416– 575
HE†	61	87	110	302	398	283–317	319– 416
JH*‡	23	90	123	170	113	160–203	113– 183
NE§	22	74	94	236	186	228–244	177– 195
				Normolipidemic relatives of patients with familial hypertriglyceridemia			
GH*‡	24	71	113	181	54	161–210	95– 144
IE§	28	72	91	236	150	232–240	124– 150
				Familial combined hyperlipidemia			
AR*	53	70	102	246	196	188–288	141– 501
CT*¶	54	114	141	293	174	240–309	174– 402
DT*¶	52	81	125	253	165	197–300	117– 262
ET*	60	78	128	242	152	236–313	152– 408
JU*‖	62	87	124	220	690	190–256	203–1100
GG	68	70	100	224	616	186–372	425– 770
BP	56	77	104	302	531	213–310	354– 708
SK	51	76	107	271	274	263–341	212– 389
BJa	34	80	103	294	885	201–321	584–1381
PP	64	70	96	290	212	273–312	191– 297
				Normolipidemic controls			
(n = 18)	44 ± 4 (23–66)	78 ± 2 (62–99)	101 ± 3 (83–126)	228 ± 9 (159–279)	116 ± 10 (62–159)		

Range of plasma lipid levels over several years of observation is indicated. Control data is reported as mean ± SEM with the ranges indicated in parentheses.

*Patients studied in Seattle.

†‡§¶Denote brothers of same family. Patients TaE and TuE are monozygotic twins.

‖JU was treated with furosemide, 40 mg/day, during the study. On this drug his plasma triglycerides were between 690 and 1100 mg/dl; when not on furosemide, his triglycerides varied between 203 and 320 mg/dl.

only elevated cholesterol (phenotype IIa). These more stringent criteria were included to eliminate, as far as possible, the families in which differentiation between FHTG and FCHL was uncertain.

Normolipidemic nonobese male subjects (age range, 23–66 yr, n = 18) were studied at both centers. In addition, we studied two consistently normolipidemic brothers of patients with FHTG. One of the patients was treated with furosemide; otherwise, none of the subjects studied had been treated with drugs or diets known to affect lipid metabolism. Cholecystograms and/or examinations with ultrasound had not shown gallstones or cholecystopathy in any of the individuals studied. The protocol was approved by the Committees on Human Research of the Karolinska Institute and the University of Washington, Seattle; informed consent was obtained from each subject.

Experimental Procedure. The subjects were hospitalized in a metabolic ward during the study and given a standardized diet of natural type for 4–7 days before and during the investigation period (cf. ref. 13). Constant body weight was maintained, and the intake of cholesterol was about 200 mg/day in each subject. Five microcuries (1 Ci = 37 GBq) each of [24-^{14}C]cholic acid (CA; 3α,7α,12α-trihydroxy-5β-cholanic acid) and [24-^{14}C]CDCA (New England Nuclear) as sodium salts dissolved in water were taken orally by the subjects in the morning before breakfast. Four to five samples of duodenal bile were collected after a 12- to 14-hr overnight fast at intervals of 1 to 3 days. Cholecystokinin octapeptide (Kinevac, Squibb) was administered i.v., and ≈5 ml of concentrated duodenal bile was obtained through a thin polyvinyl tube. A portion of the bile was immediately extracted for biliary lipid determination (see below). Venous blood samples were drawn several times during the period of study and analyzed for cholesterol, triglyceride, and lipoprotein pattern. Serum bile acid concentration in response to a standardized meal was determined as described (17).

Methods. Serum cholesterol and triglycerides were measured with a Technicon AutoAnalyzer (Technicon). Lipoprotein phenotyping was done according to World Health Organization (18) as described (13).

Measurements of Bile Acid Kinetics. After hydrolysis of the duodenal bile samples, the deconjugated bile acids were extracted and separated by thin-layer chromatography. The specific radioactivity was determined using gas/liquid chromatography and liquid scintillation counting. On the basis of the specific radioactivity curve from 4 or 5 time points, the fractional turnover rate, pool size, and synthesis of CA and CDCA were determined as outlined by Lindstedt (19). Further details of the method have been given (13).

Determination of Biliary Lipid Composition. The concentrations of bile acids, cholesterol, and phospholipids in duodenal bile were determined as described (20). The biliary lipid composition was expressed as a molar percentage of total biliary lipids, and the saturation of bile with cholesterol was calculated using the solubility limits of Carey and Small (21) assuming a total biliary lipid composition of 10 g/dl.

Analysis of Serum Bile Acids. After clotting blood at room temperature, we obtained serum by centrifugation and then froze it at −20°C for later analysis. Serum concentrations of CA and CDCA were analyzed by GC/MS using ^{2}H-labeled internal standards as described (17).

Statistical Analysis. Data are presented individually or as means ± SEM. Significances of differences between groups were tested with Wilcoxon's rank sum test, and correlations are expressed as Spearman's rank correlation coefficient, R_s.

RESULTS

Especially in FCHL, variation of lipoprotein phenotype occurs with time (5), and some patients were normotriglyceridemic during the study (Table 1). In spite of phenotypic resemblance, patients with FCHL and FHTG displayed a marked difference in bile acid kinetics (Table 2). Patients with FCHL had synthesis rates and fractional catabolic rates (FCR) of CA and CDCA that were similar to those seen in normal subjects. One subject with FCHL treated with furosemide, which elevated plasma triglyceride levels, had enhanced bile acid synthesis rates. In contrast, individuals with FHTG all displayed a clearly increased rate of bile acid synthesis ($P < 0.001$), particularly of CA ($P < 0.001$), but also of CDCA ($P < 0.001$). In addition, FHTG patients had an increased fractional catabolic rate (FCR) of both bile acids ($P < 0.001$). Note particularly the difference between two brothers that were studied, JH and GH. For several years before the study, JH had displayed hypertriglyceridemia intermittently, whereas GH had always been normolipidemic. As FHTG is usually expressed between ages 20–30 (1, 2), this implies that JH, but probably not GH, carried the gene for FHTG. During this study both brothers were normotriglyceridemic, but JH had about twice his brother's production rate of bile acids (Table 2).

Because bile acid production decreases with increasing age (20), we analyzed our data according to age distribution of the patients. Fig. 1 shows that FHTG was associated with a clearly increased bile acid production rate, regardless of age. Furthermore, for a given pool size of CA, FHTG exhibited an increased FCR (Fig. 2); similar data were obtained for CDCA (data not shown).

The consistently high production rate, as well as the increased FCR, suggested that a major abnormality of the regulation of bile acid metabolism is present in FHTG. Because bile acid production rate is regulated by the amount of bile acids returning to the liver in the portal vein (22), we estimated the fasting and postprandial inflow of bile acids in three FHTG subjects. This was achieved by determining the serum bile acid concentrations, which are known to reflect the concomitant portal venous concentrations (23). Fig. 3 shows that the fasting concentrations of bile acids were normal in the three patients with FHTG, whereas the response in serum bile acids to a standardized meal was lower than that seen in controls. Also the areas under the curves were lower in FHTG ($P < 0.05$). The pattern observed in this preliminary study was relatively similar to that seen in healthy subjects during cholestyramine feeding (17), a treatment that is associated with decreased bile acid return, increased bile acid loss, and compensatory increase in bile acid synthesis (12). These findings would thus indicate inefficient intestinal uptake of bile acids in FHTG.

Deficient bile acid return to the liver in FHTG might also result in secretion of bile supersaturated with cholesterol (24). The biliary lipid composition (in molar %—cholesterol, 5.5 ± 0.8; bile acids, 73.6 ± 3.2; and phospholipids, 20.8 ± 2.4) and cholesterol saturation (81 ± 7%) of fasting gallbladder bile of patients with FHTG were, however, not different from those of controls (in molar %—cholesterol, 5.6 ± 0.3; bile acids, 73.9 ± 0.9; and phospholipids, 20.5 ± 0.7; cholesterol saturation was 84 ± 4%).

DISCUSSION

This study clearly demonstrated that hypertriglyceridemia due to FHTG, but not to FCHL, is linked to defective bile acid metabolism, providing an explanation for the apparently conflicting reports on bile acid metabolism in hypertriglyceridemia (for review, see ref. 12). In our previous studies (13, 25), ≈50% of patients with pure hypertriglyceridemia (type IV phenotype) have overproduced bile acid. We recently

Table 2. Bile acid kinetics in patients with hereditary forms of hypertriglyceridemia

	Pool size, mg		Synthesis, mg/d			FCR, d^{-1}	
	CA	CDCA	CA	CDCA	Total	CA	CDCA
			Familial hypertriglyceridemia				
WM*	973	801	872	413	1285	0.90	0.52
BJo	850	522	724	384	1108	0.85	0.74
TaE†	795	405	888	293	1181	1.12	0.72
TuE†	1515	315	1117	233	1350	0.74	0.74
HE†	959	720	589	299	888	0.61	0.42
JH*‡	1355	990	1043	509	1552	0.77	0.51
NE§	498	547	612	394	1006	1.23	0.72
			Normolipidemic relatives of patients with familial hypertriglyceridemia				
GH*‡	1453	976	520	292	812	0.36	0.30
IE§	481	391	425	391	816	0.88	0.84
			Familial combined hyperlipidemia				
AR*	2141	1508	214	255	469	0.10	0.17
CT*¶	1360	1217	265	162	427	0.20	0.13
DT*¶	929	818	146	130	276	0.16	0.16
ET*	1216	1258	413	255	668	0.34	0.20
JU*‖	1966	1030	806	388	1194	0.41	0.38
GG	225	379	97	80	177	0.43	0.21
BP	268	322	130	127	257	0.49	0.39
SK	585	532	279	220	499	0.48	0.41
BJa	593	1134	387	329	716	0.65	0.29
PP	405	270	218	141	359	0.54	0.52
			Normolipidemic controls				
(n = 18)	777 ± 97 (132–1404)	609 ± 69 (172–1156)	288 ± 33 (76–504)	183 ± 18 (56–348)	472 ± 48 (132–852)	0.45 ± 0.06 (0.15–0.88)	0.35 ± 0.04 (0.16–0.60)

Control data is reported as mean ± SEM with the ranges indicated in parentheses.
*Indicates patients studied in Seattle.
†‡§¶Denote brothers of same family. Patients TaE and TuE are monozygotic twins.
‖This patient was taking furosemide, which increased plasma triglyceride levels.

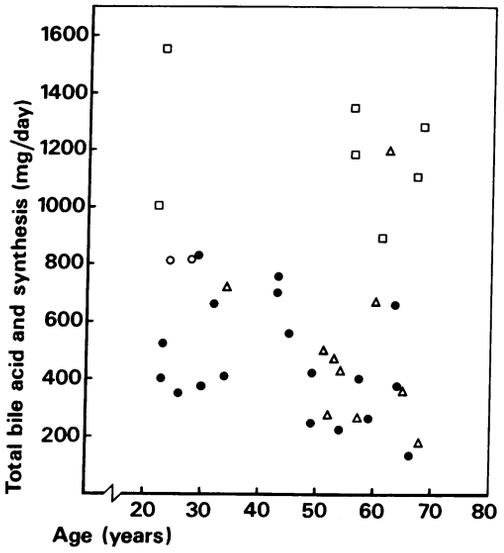

FIG. 1. Total bile acid synthesis in relation to age. Healthy controls (●), normolipidemic relatives of patients with FHTG (○), patients with FCHL (△), and patients with FHTG (□). Bile acid synthesis was negatively correlated with age in controls ($R_s = -0.49$, $P < 0.05$).

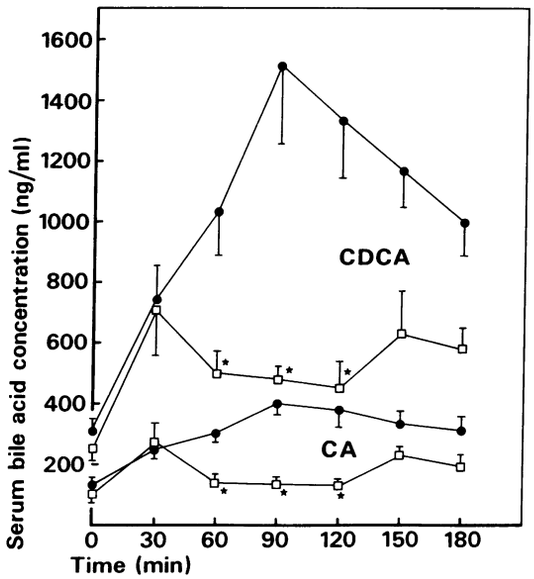

FIG. 3. Serum levels of CA and CDCA after ingestion of a standardized meal (17) in three patients with FHTG (□; WM, TaE, and JH) and five healthy controls (●), means ± SEM. * indicates statistical significance at $P < 0.05$.

demonstrated that type IV patients with high rates of bile acid synthesis also had an elevated VLDL triglyceride production (13); this subgroup probably represents FHTG, as this disorder is generally linked to VLDL triglyceride overproduction (6). In agreement with our results, Beil *et al.* (26) did not find consistent bile acid overproduction when hypertriglyceridemia was due to assumed FCHL.

The high production rate of bile acids seen in hypertriglyceridemic individuals with FHTG was also seen in an individual presumably carrying the gene, but not expressing hypertriglyceridemia at the time of study. This would imply that (*i*) bile acid overproduction may be a marker for the FHTG gene, and (*ii*) the defect in bile acid metabolism is more closely related to the basic molecular defect in these patients than the hypertriglyceridemia. Obviously, more detailed studies of patients with FHTG are needed to support these hypotheses. Considering the complexity of triglyceride metabolism, it is also probable that FHTG is a heterogenous disorder, with various metabolic abnormalities in different families. Some possible pathogenetic mechanisms can now be discussed, however.

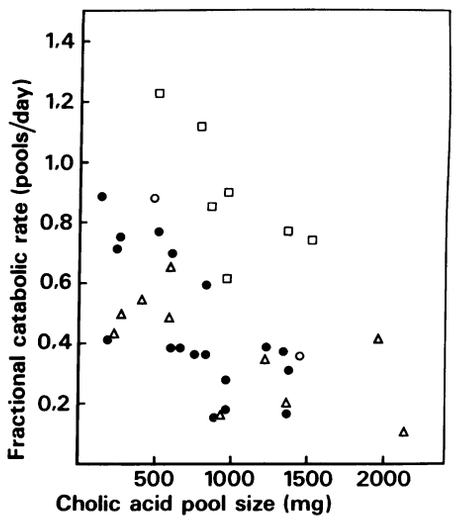

FIG. 2. Kinetics of CA in hereditary forms of hypertriglyceridemia: FCR in relation to pool size. Healthy controls (●), normolipidemic relatives of patients with FHTG (○), patients with FCHL (△), and patients with FHTG (□). FCR was negatively correlated with pool size in all groups (controls, $R_s = -0.76$; FCHL, $R_s = -0.69$; and FHTG, $R_s = -0.71$).

The probable explanation for the finding of increased FCR and enhanced production rate of bile acids in FHTG with normal-to-enlarged pool size is a deficient intestinal handling of bile acids. Uptake of bile acids secreted into the intestine occurs by at least two different mechanisms—passive, nonionic diffusion along the entire intestine, and active, probably carrier-mediated, uptake predominantly in the distal ileum (27). As judged from the postprandial response in serum bile acid concentrations, FHTG patients have a normal passive uptake of bile acids [resulting in the early (30-min) rise], whereas the active uptake [contributing to the late (90-min) peak] appears deficient. That this is the consequence of reduced function of the carrier-mediated bile acid uptake in these individuals is a tempting speculation. More detailed knowledge of the normal process of active (receptor-mediated?) absorption of bile acids in humans is needed before this hypothesis can be tested. However, at least three pieces of evidence from previous studies support the concept of deficient intestinal uptake of bile acids in some subjects with hypertriglyceridemia. (*i*) Fasting serum concentration of CA is frequently lower than would be predicted from the pool size in type IV hyperlipoproteinemia (28). (*ii*) Patients with this lipoprotein pattern tend to retain orally given CA within the bile acid pool to a lesser extent than normals (29). (*iii*) Treatment of type IV patients with cholestyramine does not stimulate bile acid production further, indicating that feedback inhibition is already absent in the basal situation (30).

Interruption of the enterohepatic circulation of bile acids results in an increased production rate of VLDL triglycerides (14, 15, 31). The exact mechanism responsible for this phenomenon is unknown, but increased activity of the hepatic enzyme, phosphatidic acid phosphatase, presumed to be rate-limiting in triglyceride biosynthesis, has been reported in the rat (cf. ref. 12). Interestingly, an increase in VLDL triglyceride production rate does not generally occur in patients with type IV hyperlipoproteinemia during choles-

tyramine treatment (14). An attractive hypothesis states that deficient intestinal reabsorption of bile acids is a primary event in FHTG, which results in enhanced synthesis rate of bile acids and increased production of VLDL triglycerides. Factors—such as body weight, age, sex, and diet, etc.—that might further influence triglyceride synthesis or influence the rate of elimination of this load of endogenous plasma triglycerides, would then finally determine the degree of hypertriglyceridemia.

In conclusion, our study gives evidence that FHTG, but not FCHL, is characterized by a bile acid metabolic defect, resulting in an increased rate of bile acid synthesis; this may lead to the subsequent development of hypertriglyceridemia. Although discussion of mechanism remains speculative, testable hypotheses may now be formulated regarding a specific defect in this group of FHTG patients.

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