

Bile Acid Kinetics in Relation to Sex, Serum Lipids, Body Weights, and Gallbladder Disease in Patients with Various Types of Hyperlipoproteinemia

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ABSTRACT Bile acid kinetics were determined in 15 normolipidemic and 61 hyperlipidemic subjects with the aid of [^{14}C]cholic acid and [^3H]chenodeoxycholic acid. The diet was standardized and of natural type. The total bile acid formation was within normal limits in patients with hyperlipoproteinemia types IIa and IIb. On the average the production of cholic acid (C) represented less than 50% of the total bile acid synthesis in both groups. The corresponding value recorded for the controls was $64 \pm 2\%$ (mean \pm SEM). The synthesis of C in hyperlipoproteinemia type IIa was significantly below normal. Of the 27 patients with the type IV pattern, 18 had a synthesis of C and C + chenodeoxycholic acid (CD) that exceeded the upper range recorded for the controls. In these subjects the C formation represented $73 \pm 3\%$ of the total bile acid synthesis. Similar findings were also encountered in the five patients with the type V lipoprotein pattern studied. The bile acid pool size of the 11 patients with hyperlipoproteinemia type IV, who had been cholecystectomized or suffered from cholelithiasis, was 900 mg smaller on the average than that of the other subjects with the same type of hyperlipoproteinemia. However, the pool size in the former subjects still tended to be higher than that of the control subjects without evidence of gallbladder "disease". In all groups of subjects the formation of bile acids tended to be higher in the male than in the female subjects. Bile acid synthesis showed no linear correlation to actual body weight, relative body weight, or body surface area. A moderate weight reduction in five patients (one with type IIb and four with type IV pattern) was followed by a 50% reduction of the C and CD synthesis.

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INTRODUCTION

Hyper- β -lipoproteinemia (type II)¹ and hyperpre- β -lipoproteinemia (type IV) are the two types of abnormal lipoprotein pattern most commonly encountered (1). Several reports demonstrate that the two disorders differ with regard to the homeostatic control of cholesterol metabolism. Kottke (2), Einarsson and Hellström (3), and Wollenweber and Stiehl (4) studied the turnover of cholic acid (C) and chenodeoxycholic acid (CD), and demonstrated that the formation of bile acids is low in patients with the type II as compared to those with the type IV lipoprotein pattern. In accordance with these observations, the fecal excretion of bile acids was found to be higher in hypercholesterolemic subjects when the hypercholesterolemia is associated with hypertriglyceridemia (5, 6).

The mechanism(s) behind the different bile acid turnovers in hyperlipoproteinemia types II and IV is not known. The present investigation is an extension of the previous one (3) and is aimed to characterize the bile acid kinetics in normolipidemic controls and in patients with four types of hyperlipoproteinemia under the same dietary conditions. Particular attention was paid to the possibility of sex differences and the effects of obesity and gallbladder disease known to influence the bile acid excretion (7) and the bile acid pool size (8-10), respectively.

METHODS

The subjects. The study comprised 76 subjects, 35-67 yr of age. Findings from some of the patients (4 with type

¹ *Abbreviations used in this paper:* C, cholic acid; CD, chenodeoxycholic acid; D, deoxycholic acid; GBD, gallbladder disease; LDL, low density lipoprotein; TFA, trifluoroacetic esters; type II, hyper- β -lipoproteinemia; type IV, hyperpre- β -lipoproteinemia; VLDL, very low density lipoproteins.

TABLE I
Basal Data about the Patients Presented as Number of Patients or Mean \pm SEM*

Type of hyperlipoproteinemia	Sex		Age yr	Body wt kg	Body wt			Previous history, present symptoms§			
	F	M			% of ideal‡	>110%	>120%	CDH	DM	IC	GBD
Controls	8	7	54.1 \pm 2.3	71.4 \pm 2.7	104 \pm 3.3	3	1	7	2	—	4
IIa	12	5	56.2 \pm 1.2	64.4 \pm 2.2	97 \pm 2.9	3	1	9	—	2	3
IIb	6	6	54.2 \pm 3.0	69.7 \pm 2.6	102 \pm 3.1	3	—	8	—	—	2
IV	4	23	51.7 \pm 1.7	81.5 \pm 1.7	111 \pm 0.2	13	6	13	3	—	11
V	3	2	49.8 \pm 5.4	73.7 \pm 8.6	104 \pm 8.0	2	2	2	1	1	3
Total	33	43	53.2 \pm 1.1	72.0 \pm 0.7	104 \pm 2.3	24	10	39	6	3	23

* The data on part of the subjects were included in a previous report (3).

‡ Calculated as [wt (kg)/length (cm) - 100] \times 100.

§ CDH, coronary heart disease; DM, diabetes mellitus; IC, intermittent claudication; GBD, gallbladder disease (cholelithiasis, cholecystitis, cholecystectomy).

|| Significantly different from controls, $P < 0.005$.

IIa, 4 with type IIb, 10 with type IV, and 3 with type V lipoprotein pattern) have been reported previously (3). The subjects were separated into five groups on the basis of their serum lipoprotein pattern (11). The normolipidemic group contained four female and eight male patients and three female members of the staff. The hyperlipidemic patients were those consecutively admitted because of hyperlipoproteinemia at the time of this investigation. Not included in the study are a few patients with hyperlipoproteinemia type III, as well as patients with intestinal, liver, and kidney disease, heart inc ompensation, hyper- and hypothyroidism, and those addicted to alcohol and narcotics. Six subjects had a mild diabetes mellitus adequately controlled by sulphonylurea or dietary regimen. Some subjects were on continuous treatment with digitalis, diuretics, and/or nitrate preparations and the therapy was kept unchanged during

the study. None of the subjects had been treated with drugs or diets known to interfere with the hyperlipoproteinemia during the months preceding this investigation. All subjects underwent a physical and laboratory investigation; an oral cholecystography was included for most of the noncholecystectomized subjects. Clinical diagnoses and basal data are listed in Table I. The patients with hyperlipoproteinemia type IV differed from the controls with regard to actual but not to relative body weight.

The serum lipids and lipoprotein pattern as evidenced by electrophoresis on agarose are summarized in Table II. Hyperlipoproteinemia type IIa was more common among the women (12 out of 17 patients) and most (23 out of 27) of the patients with the type IV pattern were men. None of the patients had a broad β -lipoprotein band.

Experimental procedure. The patients were hospitalized

TABLE II
Serum Lipids in Fasting Serum (Mean \pm SEM) and Incidence of Impaired Glucose Tolerance*

Type of hyperlipoproteinemia	Cholesterol mg/100 ml	Triglycerides mg/100 ml	Lipoprotein pattern			Chylomicron	Abnormal glucose tolerance n
			α	pre- β	β		
			%				
Controls	225 \pm 7	136 \pm 12	29 \pm 2.3	18 \pm 2.1	53 \pm 1.6	—	3
IIa	380 \pm 13‡	139 \pm 9	21 \pm 1.5§	11 \pm 1.5§	68 \pm 1.9‡	—	1
IIb	328 \pm 7	294 \pm 23‡	16 \pm 1.8	22 \pm 3.3	62 \pm 2.1	—	1
IV	240 \pm 9	369 \pm 26‡	15 \pm 1.2‡	41 \pm 2.3‡	44 \pm 1.7	—	10
V	285 \pm 33§	1,922 \pm 160‡	13 \pm 4.0	64 \pm 8.0‡	23 \pm 8.0‡	+	1

* The data on part of the subjects were included in a previous report (3).

‡ Significantly different from controls, $P < 0.001$.

§ Significantly different from controls, $P < 0.05$ or < 0.02 .

|| Significantly different from controls, $P < 0.01$ or < 0.005 .

during the study; the members of the staff remained at work, which was essentially sedentary. All subjects were interviewed about their dietary habits by a dietitian and their caloric requirements were estimated. For 4-7 days before and during the experimental period, the subjects were maintained on a standardized diet of natural type. The caloric intake, calculated from standard foodstuff tables,² was adjusted to keep the body weight constant. About 40, 21, and 39% of the calories were supplied as fat, protein, and carbohydrate, respectively. Most of the fat contained saturated fatty acids. The major part of the carbohydrates was given as starch. In each subject the intake of cholesterol was less than 200 mg/day.

[¹⁴C]C (4 μ Ci) and [³H]CD (15 μ Ci) as sodium salts were dissolved in water and taken orally by the subjects in the morning before breakfast. Four samples of duodenal bile were collected from each subject at 2- to 4-day intervals. Cholecystokinin was administered intravenously and 5-10 ml of concentrated duodenal bile was obtained through a thin polyvinyl tube. The specific radioactivities of C and CD were determined in each sample. Venous blood samples were drawn four to six times during the experimental period and analyzed for cholesterol, triglyceride, and lipoprotein pattern.

Material. [^{24-¹⁴C}]C (138 μ Ci/mg) was obtained from New England Nuclear Corp., Boston, Mass. The radiochemical purity was checked by autoradiography of thin-layer chromatograms. Randomly tritiated CD (specific radioactivity, 40 μ Ci/mg) was a gift from Dr. H. Danielsson, Karolinska Institutet, Stockholm. It had been prepared by exposing 500 mg of CD to 2 Ci of tritium gas at room temperature for 4 wk according to Wilzbach (13). The material then underwent repeated saponification and extraction procedures followed by repeated purifications by reversed phase-partition chromatography. Before use, part of the material was again subjected to reversed phase-partition chromatography and then diluted with unlabeled CD to give a substance of desired specific radioactivity. Cholecystokinin was obtained from the Gastrointestinal Hormone Research Group, Chemical Department, Karolinska Institutet, Stockholm.

Methods. Serum cholesterol was measured using a Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) and triglycerides by the method described by Laurell (13). The serum lipoproteins were separated by electrophoresis on agarose gel. The various bands were stained with Sudan black and evaluated quantitatively by densitometry (3). The upper normal limit for the pre- and the β -lipoproteins was set at 25 and 60%, respectively. The corresponding values for serum lipids were 270 mg/100 ml (total cholesterol), 210 mg/100 ml (β -cholesterol, determined in part of the patients), and 180 mg/100 ml (triglycerides). A creamy top layer of serum samples left at 4°C overnight was used as evidence of the presence of chylomicrons. Glucose tolerance was assessed with an intravenous glucose tolerance test (14). A *k*-value below 0.9 was considered abnormal. The diet was analyzed with regard to cholesterol content essentially as described in a previous paper (15).

The duodenal bile samples were hydrolyzed with N KOH in closed steel tubes for 12 h at 110°C. The deconjugated bile acids were extracted with ethyl ether and the specific activities of C and CD were determined by one or two methods. With the technique described previously (3),

² Abramson, E. 1971. Kosttabell. Läromedelsförlagen AB, Stockholm, Sweden.

the bile acids were separated into di- and trihydroxycholeanoic acid fractions by reversed phase-partition chromatography. C was recrystallized before the determination of radioactivity. The dihydroxy derivatives were analyzed by gas-liquid chromatography of the trifluoroacetic esters (TFA) of their methylesters. The radioactivity was assessed assuming that all tritium was localized to CD (16).

With the other method, the methylesters were separated by thin-layer chromatography on plates coated with silica gel (thickness 0.25 mm). The chromatograms were developed in trimethylpentane:ethyl acetate:acetic acid (10:10:2, vol/vol). The gel containing the bands with C and CD were scraped off separately. C was recovered by three extractions with methanol. To remove material interfering with the TFA formation, the gel corresponding to CD was placed on the top of silicic acid columns (height 3 cm, ID 0.5 cm) subsequently eluted with ethyl acetate. The solvents were evaporated, the TFA derivatives prepared, and the specific radioactivity determined as above. Further details about the methods used will be found in a previous paper (3).

The half-life, pool size, and turnover of C and CD were determined as outlined by Lindstedt (17). The correlation coefficients for the specific activity decay curves recorded for both C and CD averaged 0.98 ± 0.01 (mean \pm SEM).

Due to nonspecific losses of tritium, the pool size and turnover of CD will be slightly overestimated with the method used. To evaluate this error, CD labeled either uniformly with ³H or by ¹⁴C at the 24th carbon atom was administered simultaneously to five subjects who subsequently underwent the same experimental procedure as above (18). The values obtained for the pool size and turnover of CD with the former tracer exceeded those with the latter by 14 ± 2 and $17 \pm 2\%$ (means \pm SEM), respectively. Almost no differences were recorded for the half-lives.

Statistics. Data are presented as means \pm SEM. The significance of differences between means was determined by the Student's *t* test. Linear regressions have been calculated by the method of least squares and their significances tested by estimating the correlation coefficient, "*r*". The effect of weight reduction on serum lipids and bile acid turnover was evaluated by Wilcoxon signed rank test.

RESULTS

Kinetics of C and CD in subjects without gallbladder disease (GBD, cholelithiasis, or cholecystectomy). The pool size, synthesis, and fractional turnover of C in the control subjects averaged 821 ± 129 mg, 253 ± 42 mg/day, and 0.38 ± 0.08 (mean \pm SEM), respectively (Table III). The hyperlipidemic patients did not differ from the controls with regard to the C fractional turnover but the mean C pool size was above the normal range in hyperlipoproteinemia type IV. The synthesis of C was subnormal in type IIa (150 ± 17 mg/day) and abnormally high in the type IV patients (778 ± 115 mg/day).

The controls had the smallest CD pool size (575 ± 78 mg), significantly higher values being recorded for the patients with the type IIb pattern ($1,110 \pm 113$ mg). The formation of CD averaged 131 ± 40 mg/day in the controls and 251 ± 33 mg/day in the patients

TABLE III
Pool Size, Synthesis and Fractional Turnover of C and CD (mean±SEM) in Subjects with or without GBD*

Type of hyperlipoproteinemia		C			CD			C and CD	
		Pool size	Synthesis	Turnover	Pool size	Synthesis	Turnover	Pool size	Synthesis
		mg	mg/day	day ⁻¹	mg	mg/day	day ⁻¹	mg	mg/day
Control	no. GBD (11)	821±129	253±42	0.38±0.08	575±78	131±40	0.27±0.05	1,396±191	383±47
	GBD (4)	427±165	231±65	0.65±0.12	380±75	136±21	0.38±0.06	806±316	367±99
IIa	no. GBD (14)	675±103	150±17‡	0.27±0.04	843±93	179±20	0.24±0.04	1,517±160	329±26
	GBD (3)	382±136	212±64	0.60±0.08	523±79	183±6	0.36±0.01	905±187	395±71
IIb	no. GBD (10)	868±163	183±24	0.28±0.05	1,110±113	197±31	0.19±0.02	1,978±268‡	489±49
	GBD (2)	310±11	202±45	0.65±0.14	813±127	287±7	0.36±0.12	1,122±117	380±38
IV	no. GBD (16)	1,786±264§	778±115§	0.48±0.06	866±106	251±33‡	0.33±0.04	2,653±314§	1,029±124
	GBD (11)	1,174±266	709±133	0.63±0.07	471±63¶	223±37	0.48±0.04	1,711±278§	961±149
V	no. GBD (2)	2,346±1,820	506±123	0.44±0.29	801±63	186±87	0.23±0.09	3,147±1,760	692±37
	GBD (3)	1,917±862	642±216	0.51±0.27	921±227	201±122	0.33±0.10	2,837±1,050	910±299

* The number of subjects is within parentheses; the data on part of the subjects were included in a previous report (3).

‡ Significantly different from controls without GBD, $P < 0.05$ or < 0.02 .

§ Significantly different from controls without GBD, $P < 0.01$ or < 0.005 .

|| Significantly different from controls without GBD, $P < 0.001$.

¶ Significantly different from type IV patients without GBD, $P < 0.05$ or < 0.01 .

with hyperlipoproteinemia type IV ($P < 0.05$). Although not significant on a statistical basis, the fractional turnover of CD tended to be lower than that of C in all groups of subjects.

The combined pool size of the two primary bile acids and the total bile acid turnover showed qualitative as

well as quantitative differences among the various groups (Table III). In the controls the mean pool size and the turnover of C + CD were $1,396 \pm 191$ mg and 383 ± 47 mg/day, respectively. C was the dominant bile acid, on the average exceeding CD by factors of 1.4 (pool size) and 1.9 (synthesis).

The patients with hyperlipoproteinemia types IIa and IIb were characterized by a low C:CD ratio of both bile acid pool size and synthesis. The average quotient encountered for bile acid synthesis was less than half that recorded for the controls. However, the total bile acid formation was within the normal range in both types IIa and IIb, as was the combined C and CD pool size in type IIa. This parameter was 400 mg higher on the average in the type IIb patients than in the controls (Table III).

Due to the large pool size in the patients with hyperlipoproteinemia type IV, the combined pool size of C and CD was almost twice as high as in the controls. The mean total bile acid formation averaged $1,029 \pm 124$ mg/day in the type IV patients thus exceeding that of the controls by a factor of 2.7. Accordingly, the ratios for the C:CD pool sizes and turnovers tended to be above the normal range.

Bile acid kinetics in subjects with GBD. Altogether 11 patients with hyperlipoproteinemia type IV were cholecystectomized or had cholelithiasis. As compared with the other patients with the type IV lipoprotein pattern, those with GBD had higher fractional turnovers and smaller pool sizes of CD (Table III). Although not significant, a similar tendency was observed for C. The combined pool size of C and CD was about 900 mg smaller on the average in the patients with

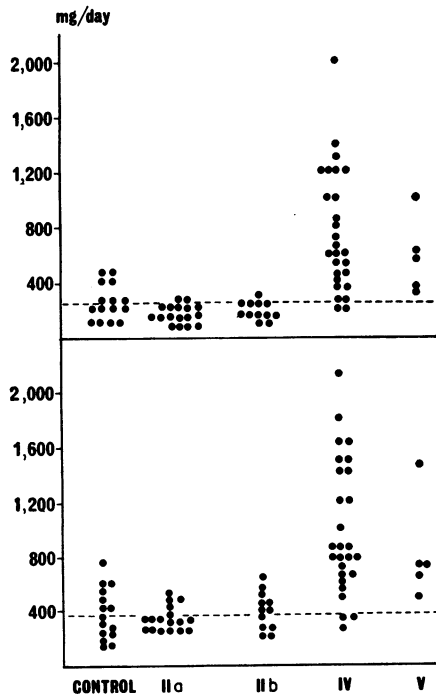


FIGURE 1 Synthesis of C (upper panel) and C + CD (lower panel). The data about some of the patients were included in a previous report (3).

TABLE IV
*C Pool Size and Synthesis Expressed as Percent of the Combined Amount of the Two Primary Bile Acids in the Total Series of Subjects**

Type of hyperlipoproteinemia	Pool size	Synthesis
Control	56±3	64±2
IIa	42±3‡	46±2
IIb	39±3	48±3
IV	67±3§	73±3‡
V	62±8	71±4

* Mean±SEM; the data on part of the subjects were included in a previous study (3).

‡ Significantly different from controls, $P < 0.05$ or < 0.02 .

§ Significantly different from controls, $P < 0.01$.

|| Significantly different from controls, $P < 0.001$.

GBD. The synthetic rate of both bile acids was not influenced by the anatomical state of the biliary tract. A tendency of a reduction of the total bile acid pool size in association with a "normal" bile acid synthesis was also observed in the other patients suffering from GBD. Since 23 of the 76 subjects investigated were cholecystectomized or had cholelithiasis, most of the discussion below will be limited to bile acid formation.

Bile acid kinetics (total series). Individual data for the synthesis of C and C + CD are demonstrated in Fig. 1. In keeping with the values obtained in subjects without GBD, the formation of C in the patients with hyperlipoproteinemia type IIa (161 ± 18 mg/day) was significantly ($P < 0.02$) lower than that recorded for the controls (247 ± 34 mg/day). The corresponding

TABLE V
*C:CD Ratio of Pool Sizes in Subjects in Whom the Ratios between the Concentrations of C:CD:D in Duodenal Bile were Determined**

Type of hyperlipoproteinemia	n	Duodenal bile	
		C:CD	C:CD:D
Controls	13	1.44±0.18	1.06±0.14:1:0.92±0.13
IIa	12	0.83±0.08	1.07±0.12:1:1.14±0.22
IIb	8	0.75±0.09‡	1.10±0.12:1:0.63±0.16
IV	15	2.70±0.47‡	1.43±0.20:1:1.35±0.19
V	2	1.30	0.90:1:0.85

* Means±SEM.

‡ Significantly different from C:CD ratio of bile acids in duodenal bile, $P < 0.05$ or < 0.02 .

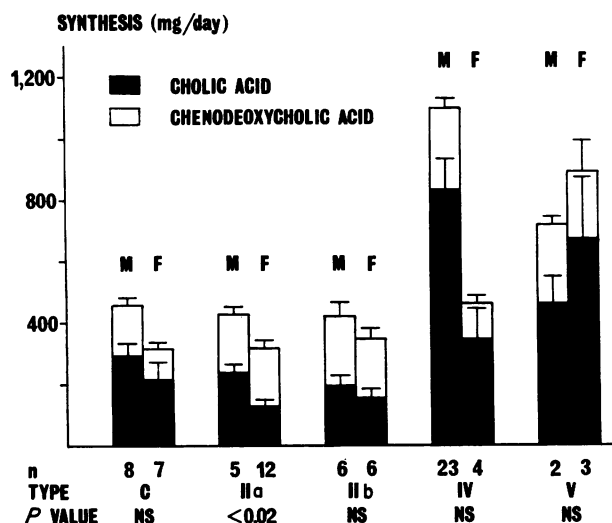


FIGURE 2 Synthesis of C and CD in the male (M) and female (F) control (C) and hyperlipidemic subjects (mean, SEM). The data on part of the patients were included in a previous report (3).

results encountered for the patients with type IV (762 ± 86 mg/day) and type V (587 ± 128 mg/day) exceeded those of the controls ($P < 0.001$ and < 0.01 , respectively). The total bile acid formation was within the normal range (379 ± 47 mg/day) in hyperlipoproteinemia type IIa and IIb but abnormally high in type IV ($1,001 \pm 94$ mg/day) and type V (822 ± 173 mg/day). In 18 of the 27 patients with type IV, the total bile

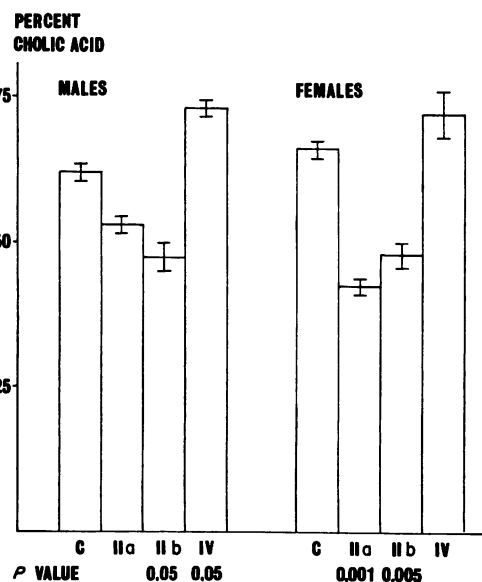


FIGURE 3 C synthesis expressed as percent of total bile acid formation in male and female control and hyperlipidemic subjects. The data on part of the patients were included in a previous report (3).

TABLE VI
Serum Lipids and Body Weights in Men and Women*

Type of hyperlipoproteinemia	Serum cholesterol		Serum triglycerides		Body wt	
	M	F	M	F	M	F
	<i>mg/100 ml</i>		<i>mg/100 ml</i>		<i>kg</i>	
Control (7 M, 8 F)	226±12	224±8	162±9	109±19‡	79±3	64±3§
IIa (5 M, 12 F)	338±29	398±12	131±21	140±9	68±2	63±3
IIb (6 M, 6 F)	321±4	326±15	290±39	284±26	76±2	64±3§
IV (23 M, 4 F)	244±10	236±40	381±29	301±27	82±2	77±2

* Mean±SEM.

‡ Significantly different from M, $P < 0.05$.

§ Significantly different from M, $P < 0.01$ or < 0.005 .

acid synthesis was higher than the highest value recorded for the controls (Fig. 1).

C constituted $64 \pm 2\%$ of the total bile acids produced in the controls and on the average, less than 50% of those synthesized in the patients with hyperlipoproteinemia type II (Table IV). The subjects with hyperlipoproteinemia type V were similar to those with the type IV pattern. C represented about 70% of the total bile acid production in those two groups.

Bile acid composition in duodenal bile. Aliquots of saponified specimens of duodenal bile from some of the subjects were analyzed by GLC without further fractionation (Table V). Disregarding the small peaks corresponding to lithocholic acid, the gas chromatograms demonstrated that on the average, 35.6, 33.6, and 30.8% of the biliary bile acids in the controls were made up of C, CD, and deoxycholic acid (D), respectively. The composition of the bile acids in duodenal bile of the hyperlipidemic patients was essentially the same as in the controls in the presence as well as in the absence of GBD.

The C:CD ratios for the concentrations of bile acids in duodenal bile were not the same as the corresponding ratios for pool sizes (Table V). Whereas the former ratios tended to be higher than the latter in hyperlipoproteinemia type II, they were lower in hyperlipoproteinemia type IV. A similar tendency, although less pronounced, was observed in the controls.

Bile acid synthesis in relation to sex. In all groups except the small one with the type V lipoprotein pattern, the bile acid synthesis tended to be higher in the male than in the female subjects (Fig. 2). The difference between the sexes was significant with regard to CD in the controls ($P < 0.05$), for C ($P < 0.01$), and C and CD ($P < 0.02$) in hyperlipoproteinemia type IIa. The formation of C in the men with the type IV pattern differed significantly ($P < 0.005$) from those of the male controls. The daily synthesis of CD in the women with hyperlipoproteinemia types IIa and IIb exceeded that of the female controls ($P < 0.005$ and < 0.02 , respectively). The C production in the four women with the type IV pattern was within the normal female limit.

Although it is not significant on a statistical basis in the males with hyperlipoproteinemia type IIa, the synthesis of C represented a subnormal fraction of the total bile acid formation in both sexes. The corresponding figure calculated for the males with hyperlipoproteinemia type IV exceeded the range encountered for the male controls (Fig. 3).

The men tended to be heavier than the women. The difference reached significant levels in the controls

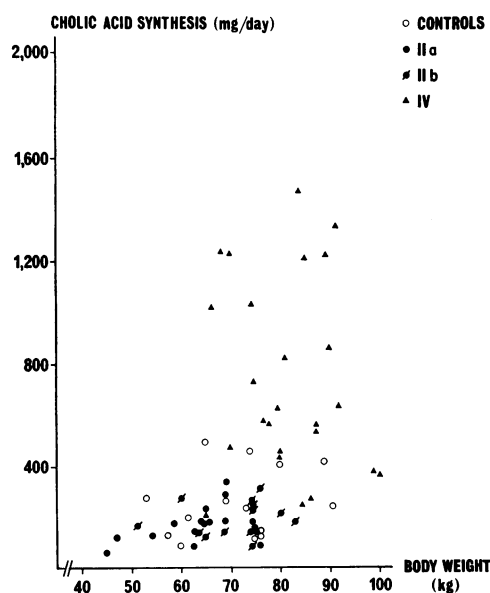


FIGURE 4 Synthesis of C in relation to actual body weight. The data on part of the subjects were included in a previous report (3).

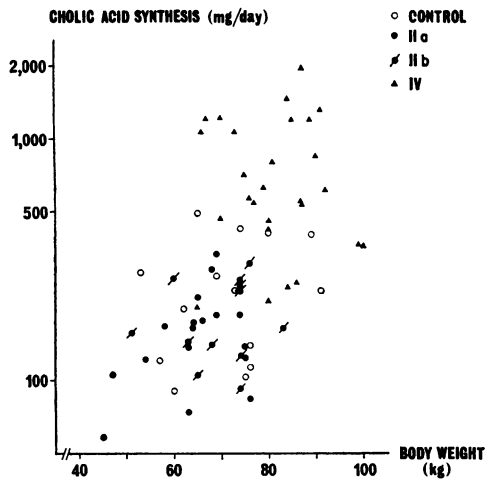


FIGURE 5 Synthesis of C (logarithmic value) in relation to actual body weight. The data on part of the subjects were included in a previous report (3).

and in the patients with hyperlipoproteinemia type IIb. The two sexes did not differ with regard to serum cholesterol level but the serum triglyceride concentration was higher in the male than in the female controls (Table VI).

Bile acid synthesis in relation to body weight and serum lipids. The formation of C, CD, and C + CD was plotted against actual weights, relative weights (percentage of ideal weights), overweight and body surface area. The three latter parameters showed no correlation to bile acid synthesis. C production was slightly correlated to actual body weight ($r = 0.32$, $P < 0.05$) in the group comprising both controls and subjects

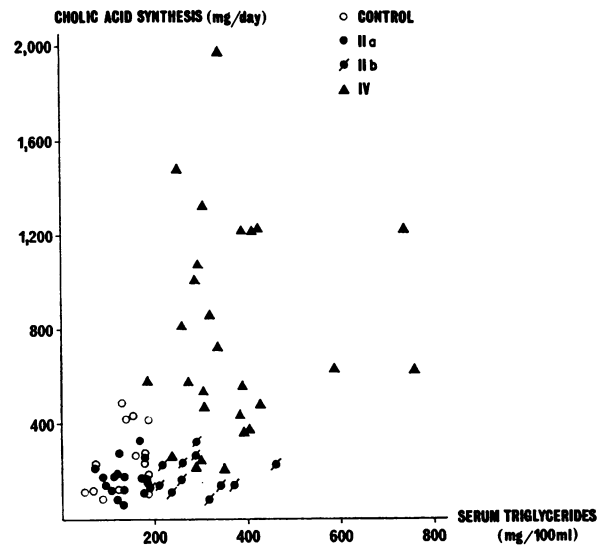


FIGURE 6 Synthesis of C in relation to serum triglyceride level. The data on part of the subjects were included in a previous report (3).

with the types IIa and IIb lipoprotein pattern. On combining all subjects except those with hyperlipoproteinemia type V, the relation between C and C + CD synthesis appears to be definitely nonlinear over a body weight of 75–80 kg, i.e., the lower limit for many patients with hyperlipoproteinemia type IV (Fig. 4). However, as shown in Fig. 5 there was a highly significant correlation between the actual body weight and the logarithmic values of the C and C + CD formation. In the complete series of subjects (type V excluded), the triglycerides were correlated to the pro-

TABLE VII
Body Weights, Serum Lipids, and the Turnover of C and CD before (B) and after (A)
Weight Reduction in Five Patients with Hypertriglyceridemia

Patient	Body wt	Cholesterol	Triglycerides	C pool size	C turnover	CD pool size	CD turnover	C + CD pool size	C + CD turnover
	kg	mg/100 ml	mg/100 ml	mg	mg/day	mg	mg/day	mg	mg/day
1. B	89	180 ± 5	416 ± 47	2,190	1,217	1,160	328	3,350	1,545
A	76	180 ± 1	79 ± 20	825	337	712	183	1,537	520
2. B	66	248 ± 4	254 ± 26	3,266	1,008	1,084	195	4,350	1,203
A	61	247 ± 14	141 ± 13	481	186	488	117	969	303
3. B	67	255 ± 10	736 ± 106	2,454	1,217	595	207	3,049	1,424
A	62	286 ± 10	312 ± 47	3,507	840	435	98	3,942	938
4. B	102	287 ± 9	394 ± 8	675	361	937	312	1,612	673
A	90	229 ± 15	280 ± 35	931	259	295	89	1,226	348
5. B	76	313 ± 23	286 ± 57	1,016	307	1,300	265	2,316	572
A	64	254 ± 20	253 ± 15	1,590	175	1,210	118	2,800	293
Mean	80 ± 6	257 ± 22	417 ± 57	1,920 ± 476	822 ± 201	1,015 ± 120	261 ± 37	2,935 ± 462	1,083 ± 196
±SEM	71 ± 4†	239 ± 17	213 ± 43	1,467 ± 123*	359 ± 123*	628 ± 159*	121 ± 17‡	2,095 ± 558	480 ± 121*

* Significantly different from values encountered before weight reduction, $P < 0.05$ or < 0.02 .

‡ Significantly different from values encountered before weight reduction, $P < 0.01$ or < 0.005 .

duction of C ($r = 0.50$, $P < 0.001$, Fig. 6), CD ($r = 31$, $P < 0.01$), and C + CD ($r = 0.52$, $P < 0.001$). No correlation was found between serum cholesterol level and bile acid synthesis.

To obtain further information concerning the possible relationship between bile acid kinetics, body weights, and serum lipids, five male patients, one with hyperlipoproteinemia type IIb (no. 5, Table VII) and four with type IV lipoprotein pattern (nos. 1-4, Table VII) were re-examined after 3-26 mo when they had voluntarily reduced their weight 5-13 kg. The diets used were exactly the same on both turnover studies. Before the second study the body weights had been fairly stable (± 1 kg) for 24, 9, 3, 0.5, and 17 mo in patients 1, 2, 3, 4, and 5, respectively. In all subjects, the weight reduction was associated with a decrease in the serum triglyceride level and in the synthesis of C, CD, and C + CD. All these parameters were 50% lower on the average than in the first study (Table VII).

Bile acid synthesis in patients with abnormal glucose tolerance. Of the patients with hyperlipoproteinemia type IV, 9 had a normal, 4 a borderline, and 10 an abnormal glucose tolerance. The patients in the latter groups were heavier and tended to have higher serum triglyceride levels. No consistent differences were observed with regard to bile acid formation.

Bile acid synthesis in relation to dietary intake. The composition of the diet was almost identical in all subjects with regard to the percentages of fat, protein, and carbohydrate. The caloric intake prescribed to keep body weight constant ranged between 1,500 and 2,200 kcal/day. In the complete series of patients, the synthesis of C and C + CD was uncorrelated to the amount of calories ingested.

DISCUSSION

Several investigations with the isotope dilution technique have demonstrated that the pool size and synthesis of C in healthy subjects given diets of natural type average about 1,200 mg and 300 mg/day, respectively (8, 9, 17, 19, 20). In accordance with the results recorded for the normolipidemic controls in the present study, the synthesis of CD has been found to be about half that of C (9, 20). Similar results concerning the ratio between the synthetic rates of the two primary bile acids were reported recently by Quarfordt and Greenfield (21), who studied the metabolic fate of [^3H]cholesterol.

The radioactive CD used by Vlahcevic, Miller, Farrar, and Swell (20) was labeled with ^{14}C in the 24th carbon atom, the one employed by Danzinger, Hofmann, Thistle, and Schoenfield (9) had ^3H in the 2,4-position, whereas the one administered in the present

study was randomly marked with tritium. The similarity of the results obtained in normolipidemic subjects with the different types of labeling indicate that nonspecific losses of tritium during the enterohepatic circulation of [^3H]CD (or during the saponification procedure) are of minor importance. Further support for this view was obtained from the simultaneous administration of both [^{14}C]CD and [^3H]CD to five subjects (see Methods).

Studies of bile acid kinetics by isotope dilution technique require steady-state conditions, i.e., synthesis has to equal excretion and the bile acid pool size has to be constant during the experimental period. Since bile acid formation in animals (22-25), as well as in man (9, 26, 27), is regulated by the amount of bile acids reaching the liver by the portal vein, changes of intestinal microflora and motility may influence bile acid kinetics. To keep the patients in a steady state, they were maintained on a carefully standardized diet which was tolerated without complaints. None had received drug treatment because of hyperlipoproteinemia or antibiotics for at least 1 mo before the study.

The present study demonstrates that bile acid kinetics in hyperlipidemic subjects differ from those in controls. A consistent finding in patients with hyper- β -lipoproteinemia was the subnormal formation of C, in many cases compensated for by a slight elevation of the CD production. However, the total bile acid synthesis in hyperlipoproteinemia type IIa as well as in type IIb was within the normal range. The relationship was quite different in patients with types IV and V lipoprotein patterns. Due almost exclusively to a high C formation, the total bile acid synthesis was two to three times higher on the average than in the controls.

Apart from the difference in their serum lipoprotein pattern, the various groups of subjects differed with regard to the incidence of biliary tract "disease" (gallstone or cholecystectomy), abnormal glucose tolerance, sex, and body weight. A question of importance is whether these parameters per se influenced the bile acid kinetics.

Biliary tract disease was most common among the patients with hyperlipoproteinemia type IV (11 out of 27 patients, 41%). Previous studies (8-10) in normolipidemic subjects have demonstrated that cholesterol gallstone disease and cholecystectomy are associated with a reduction of the bile acid pool size. Similar findings were obtained in the present patients. In the subjects with the type IV lipoprotein pattern, the combined pool size of C and CD averaged 900 mg less than in the subjects with normal gallbladders. However, even in the presence of GBD, the total bile acid pool size in hyperlipoproteinemia type IV tended to exceed that of the controls without this disorder. In accord-

ance with other reports (8-10), there was no indication that GBD interferes with bile acid formation.

The high incidence of abnormal glucose tolerance (10 out of 27 patients) in hyperlipoproteinemia type IV is compatible with previous observations (1). There was no evidence that abnormal glucose tolerance was associated with specific changes of bile acid kinetics.

In the present, as in several other studies dealing with hyperlipoproteinemia (2-4, 6, 7, 28) women are overrepresented among the subjects with type II and men among those with type IV. This phenomenon may be of importance to consider since the present results indicate the existence of small sex differences in bile acid kinetics. Qualitatively in subjects with the type IIa lipoprotein pattern, the formation of C tended to be more important relative to CD in men than in women. However, although not significantly demonstrated in all subgroups, the conclusions above concerning the qualitative characteristics of bile acid turnover in the various types of hyperlipoproteinemia seem to be valid for both sexes.

Quantitatively, bile acid synthesis appeared to be somewhat higher in the males than in the females of all the four major groups studied (Fig. 2). A similar tendency for bile acid excretion to differ between men and women has been noted by Miettinen (7). It was not established whether these differences in the present subjects were secondary to differences in body weight and/or serum lipid levels (see below) as opposed to other factors.

Excessive body weight, which is often associated with hypertriglyceridemia, has been reported to be associated with increased cholesterol and bile acid synthesis (7, 29). Miettinen (7) found a positive correlation between body weight and the fecal excretion of neutral, acidic, and total steroids and concluded that cholesterol elimination calculated per kilograms body weight was of equal magnitude in hypertriglyceridemic and control subjects. The present study demonstrated that the actual body weight showed no linear relationship to bile acid turnover unless the calculations were made from the logarithmic values of the latter parameter.

Other reports indicate that the serum triglyceride concentration rather than obesity is primarily related to cholesterol and bile acid formation (6, 30). This concept is not fully supported by the present investigation as no significant relationship was found between bile acid synthesis and triglyceride levels in the separate groups of subjects. More suggestive, however, are the results in the five patients studied before and after moderate weight reduction, which resulted in a marked decrease both in the triglyceride level and in bile acid turnover. In these subjects the synthesis of C

and the total formation of bile acids were correlated to the serum triglyceride level rather than to the actual or relative body weights of the subjects. The effects of weight reduction in moderately obese normal and hyperlipoproteinemic subjects have been investigated recently (28). Obesity was found to have a modest influence on a number of metabolic variables (insulin resistance, fasting insulin and triglyceride levels, and very low density lipoprotein (VLDL)-triglyceride production rate) but did not appear to be the sole determinant of any of them. The influence of overweight on these various parameters and possibly also on bile acid and cholesterol formation may show extensive inter-individual variability.

Hyperlipoproteinemia may be due to an abnormal metabolism of the intact lipoprotein, the protein moiety or any of its lipid constituents. In patients with the type II lipoprotein pattern, the synthetic rate of low density lipoprotein, β -lipoprotein (LDL apoprotein) is within normal limits but the fractional catabolic rate of LDL is reduced and the LDL half-life prolonged (31). In patients with this disorder, the metabolic fate of the cholesterol moiety in LDL seems to parallel that of the apoprotein (29, 32).

In keeping with the findings above, the present patients with hyper- β -lipoproteinemia had a normal synthetic rate of the main cholesterol degradation products, i.e., the bile acids. Although not significant, their fractional turnover rates tended to be subnormal. However, the most impressive abnormality of bile acid kinetics was of a qualitative rather than a quantitative nature. Preliminary results (Einarsson, Hellström, and Kallner, to be published) demonstrate that the fecal excretion of neutral steroids, and thus the cholesterol balance, is within the normal range.

Additional information on the mechanism causing hyper- β -lipoproteinemia may be obtained from the therapeutic response to cholestyramine. Treatment with this drug often results in a normalization of the type II lipoprotein pattern (33). The effect is not achieved by a reduced LDL synthesis but is associated with an increased fractional catabolic rate of the LDL protein moiety (34). The severalfold increase of bile acid turnover observed in patients with hyperlipoproteinemia type II during cholestyramine treatment is due predominantly to a stimulation of C synthesis, whereby the abnormally low ratio encountered between the C and CD synthesis changes to normal (35). Thus the abnormal LDL metabolism in hyperlipoproteinemia type II appears to be closely linked to abnormal bile acid kinetics. The latter may or may not be the more fundamental defect.

The mechanisms primarily responsible for the accumulation of VLDL in hyperlipoproteinemia type IV

are not yet established. An increased formation and secretion of VLDL from the liver, has been observed (36, 37). Nikkilä and Kekki (37) also found that the inflow decreased upon administration of a clofibrate derivative, a therapeutic regimen which in this disorder is associated with a reduced bile acid formation (5, 38). However, contradictory results, indicating defects in extrahepatic mechanisms for the removal of VLDL-triglyceride rather than an overproduction of the lipoproteins, have been reported repeatedly (39, 40). If so, the formation of bile acids in hyperlipoproteinemia type IV could not be linked directly to VLDL synthesis or secretion rate. The large bile acid pool size so commonly encountered and the tendency for fractional bile acid turnover to be elevated in this disorder suggest a defect at intestinal level. Studies to evaluate this possibility are in progress.

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