

Measurement of bile acid production in hyperlipidemic man: does phenotype or methodology make the difference?

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Abstract Bile acid production has been measured in 13 studies in 10 hyperlipidemic subjects by simultaneous use of isotope dilution kinetics and chemical balance methodology. When the data of all 13 studies were averaged, the correlation between the two sets of values was negative and weak ($r = -0.01$, NS). However, when the correlations for normoglyceridemic and hyperglyceridemic subjects were examined separately, a strong positive correlation was found between the values obtained by the two methods in normoglyceridemic subjects ($r = 0.92$, $P < 0.01$) but not in hyperglyceridemic subjects ($r = 0.25$, NS). Examination of bile acid specific activity decay characteristics in eight studies, where bile was sampled up to six times within the first 24 hr after radio-labeled bile acid infusion, revealed differences in the rate of attainment of peak specific activity and in the time taken subsequently to achieve first-order kinetic decay. However, analysis of the data from these eight studies by input-output analysis yielded values for primary bile acid synthesis no different than those generated by conventional isotope dilution kinetics. Thus, bile acid production in normoglyceridemic subjects may be accurately quantitated by either isotope dilution or chemical balance methodology. Our data, as well as results from other laboratories, indicate that the values obtained are strictly comparable. On the other hand, the quantitation of bile acid production in hyperglyceridemic subjects by isotope dilution kinetics gives higher values than those obtained by chemical balance methodology, and in addition, higher values than those obtained in patients with normal plasma triglyceride levels.—Davidson, N. O., P. Samuel, S. Lieberman, S. P. Shane, J. R. Crouse, and E. H. Ahrens, Jr. Measurement of bile acid production in hyperlipidemic man: does phenotype or methodology make the difference? *J. Lipid Res.* 1981; **22**: 620–631.

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Bile acid formation is quantitatively the most important route for cholesterol degradation in man. Quantification of this key parameter of cholesterol homeostasis is critical to many areas of research, and the results have important implications in medical practice. There are presently two methods for meas-

uring bile acid synthesis in man: first, the isotope dilution method of Lindstedt (1) and second, the chemical balance method originally described in this laboratory (2). Using the isotope dilution method, workers have reported a difference in the rate of bile acid synthesis in hyperglyceridemic patients compared to normoglyceridemic (but hypercholesterolemic) subjects (3–7); the question of this difference has not been specifically addressed by investigators using chemical balance methodology. In contrast, there is disagreement as to whether the isotope dilution method gives a valid estimate of bile acid synthesis by comparison to chemical balance methods in the same patient; some workers describe a systematic overestimate by isotope dilution kinetics in comparison to results achieved by chemical balance methods (8–10), while others find that the two techniques give results that agree closely with one another (11).

The present study was carried out in ten patients with either hypercholesterolemia or hyperglyceridemia or both. It was designed to test for differences in bile acid production attributable either to phenotype or methodology; 13 studies were carried out using both methodologies simultaneously in the same patient. The results suggest that in hypercholesterolemic patients isotope dilution kinetics give a value for bile acid synthesis close to that derived by chemical balance methodology. However, in patients with hyperglyceridemia, higher values for bile acid synthesis are found by isotope dilution kinetics than by chemical balance studies; in addition, higher values

Abbreviations: TLC, thin-layer chromatography; GLC, gas-liquid chromatography; TMS, trimethylsilyl.

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are found by isotope dilution kinetics in hyperglyceridemic as compared to hypercholesterolemic patients. Nevertheless, our own data show, as does a detailed review of the literature, that bile acid production as measured by chemical balance methods is not significantly different in hypercholesterolemic and hyperglyceridemic patients.

MATERIALS AND METHODS

Patients

Ten patients were admitted to the metabolic ward of Rockefeller University Hospital. The patients were characterized on the basis of Fredrickson's (12) phenotyping as having type IIa ($n = 4$), type IIb ($n = 2$), type IV ($n = 3$), or type V ($n = 1$) hyperlipoproteinemia. For the purposes of our study, these patients were divided into two groups: those with and those without elevated plasma triglyceride levels in excess of 180 mg/dl, as defined by fasting levels on three occasions.

After review and approval of the study protocol by The Rockefeller University Institutional Review Board, informed consent was given by all patients. Relevant clinical data are presented in **Table 1**. With the exception of two patients (7 and 8), all subjects had normal gallbladder function as determined by oral cholecystography and were free from cholelithiasis. All patients were maintained on repetitive eucaloric diets—in some instances by liquid formula feeding (13, 14), in other cases with cyclic (2- or 3-day) solid food diets of measured protein, fat, and carbohydrate composition—to establish constant body weight and plasma lipid levels. Details of caloric intake and dietary sterol content are listed in Table 1. Following admission to the metabolic ward, patients generally required 14 days to establish a clinical steady state in which body weight was maintained constant ± 0.2 kg, biweekly plasma cholesterol and triglyceride determinations showed a coefficient of variation of less than 10% and 20%, respectively, with no obvious trends, and recovery of chromic oxide from the stool (15) exceeded 80%. Only after these criteria were satisfied was any study conducted.

Isotope administration

[24-¹⁴C]Cholic acid and [24-¹⁴C]chenodeoxycholic acid were purchased from Amersham-Searle Corp., Arlington Heights, IL; the specific activities were 52 mCi/mmol and 59 mCi/mmol, respectively. Each was purified upon receipt by thin-layer chromatography (TLC) using silica gel G in a solvent system of iso-

octane–ethyl acetate–glacial acetic acid 10:10:2 (v:v:v), and found to be greater than 97% radiochemically pure. Known doses of [¹⁴C]cholic acid and [¹⁴C]chenodeoxycholic acid in 1 ml absolute ethanol were added to 150 ml physiological saline and, following an overnight fast, this mixture was infused intravenously 30 min prior to the patients' usual breakfast. Patients were encouraged to maintain their usual eating habits; even those patients in whom bile aspirations were made at 4-hr intervals did not alter their eating patterns.

Technique of sample collection

On the day prior to infusion of labeled bile acids, each patient was intubated with a nasoduodenal tube that was positioned radiographically in the second portion of the duodenum where it remained for the duration of the study (5 days). Bile flow was stimulated by intravenous injection of sincalide, a synthetic preparation of the C-terminal octapeptide of cholecystokinin (Kinevac, E. R. Squibb & Sons Inc., Princeton, NJ), 0.02 μ g/kg body weight. Usually between 1 and 2 ml dark golden bile was sent to the laboratory, the remainder being returned to the patient via the tube which was then flushed with normal saline. In eight patients, early time points were examined by aspirating small volumes of bile at 4-hr intervals for the first 24 hr following infusion. In all patients, bile was aspirated at 24 hr and subsequently at 24 hr intervals up to 120 hr. In five of the eight patients in whom bile samples were collected on multiple occasions within the first 24 hr, mass determinations of cholic and chenodeoxycholic acid methyl esters by gas–liquid chromatography (GLC) indicated a total removal of only 0.49% and 0.69% of the respective pool of each primary bile acid. Bile samples were immediately added to ten volumes of absolute ethanol and refrigerated at 4°C until analyzed.

Specific activity measurements

For determination of bile acid specific activity, 2 ml of the ethanolic-bile mixture was taken into a Teflon tube and evaporated under nitrogen at room temperature to remove excess ethanol. The mixture was then taken up in 1 ml of 10 N NaOH and saponified at 15 psi for 3 hr. Ten ml of distilled water was added and the entire contents transferred to a 50-ml glass centrifuge tube. The neutral sterols were removed with three extractions of 20 ml of petroleum ether (bp 30–60°C) and the remaining lower phase was acidified to pH 1 with concentrated HCl. Bile acids were extracted into ethyl acetate (3×20 ml) and the combined extracts were evaporated. The methyl ester derivatives were prepared and the mixture was

TABLE 1. Relevant clinical data in ten patients whose daily bile acid synthesis was simultaneously quantitated by chemical balance and isotope dilution techniques

Patient	Age (yr)	Sex	Height (cm)	Weight (kg)	Relative ^a Body Weight	Diet	K ^b calories	Dietary ^c Sterols			Plasma Lipids ^d		Diagnosis ^e
								Chol.	Sito.	Cholesterol	Cholesterol	Triglycerides	
1 EL (a) ^f	50	M	183	88	106	Solid ^g	2500	107	143	391 ± 26 (15)	148 ± 20 (15)	Hypercholesterolemia, type IIa, TX, IHD,	
(b)	50	M	183	86	104	Solid	2575	139	330	361 ± 23 (10)	141 ± 23 (10)		
(c)	50	M	183	85.5	103	Solid	2575	139	330	361 ± 23	141 ± 23		
2 AM	46	M	175	81	108	Solid	2880	99	244	411 ± 180 (6)	161 ± 37 (6)	Hypercholesterolemia, type IIa, TX, IHD	
3 TF (a)	68	F	159	82	139	Formula ^h	2200	34	588	375 ± 13 (11)	213 ± 24 (11)	Combined hyperlipidemia, type IIb, TX, IHD	
(b)	68	F	159	74	125	Formula	1900	29	508	364 ± 21 (14)	172 ± 32 (14)		
4 PC	52	M	168	72	106	Solid	2700	127	284	552 ± 27 (11)	208 ± 28 (11)	Combined hyperlipidemia, type IIb, TX, IHD,	
5 GDeR	51	M	169	67	97	Formula	2400	341	262	211 ± 13 (10)	510 ± 94 (10)	Hyperglyceridemia, type IV, IHD	
6 BP	61	F	154	53	98	Solid	1650	80	198	317 ± 49 (7)	324 ± 72 (7)	Combined hyperlipidemia, type IV, IHD	
7 SB	29	M	177	83.6	109	Formula	2900	44	386	360 ± 23 (6)	153 ± 36 (6)	Hypercholesterolemia, type IIa, TX, gout, cholecystectomy	
8 AH	55	F	152	60.5	116	Solid	1900	75	247	235 ± 19 (8)	82 ± 4 (8)	Hypercholesterolemia, type IIa, IHD, cholelithiasis with functional gallbladder	
9 EC	53	F	155	74	135	Solid	2600	96	359	230 ± 7 (7)	244 ± 39 (7)	Combined hyperlipidemia, type IV	
10 PW	37	M	170	90	129	Solid	2800	159	188	280 ± 14 (8)	627 ± 54 (8)	Combined hyperlipidemia, type IV, IHD, gout	

^a Relative body weight (% ideal body weight) = [Weight (kg)/Height (cm) - 100] × 100.

^b Daily energy intake required to maintain constant body weight (1 kilocalorie = 4.184 kilojoules).

^c Daily intake in mg/day.

^d During metabolic steady state. ^e mg/dl ± S.D. (see text); number of analyses in parentheses.

^e Abbreviations: TX, tendon xanthomatosis; IHD, ischemic heart disease. Hyperlipidemic phenotypes according to Fredrickson (12).

^f Letters in parentheses refer to successive study periods in the same patient.

^g Solid food diets given in repetitive 2- or 3-day cycles and determined by analysis as furnishing the same mixture of major nutrients, cholesterol, and plant sterols each day. Study subjects 1(a), 1(b), 1(c), 2, 4, 6, 8, and 9 received 35% of their daily caloric intake as mixed food fats with a P/S ratio of 2/1; protein and carbohydrate comprised 20% and 45%, respectively. Study subject 10 received 61% daily caloric intake as fat with a P/S ratio of 4.3/1; protein and carbohydrate constituted 14% and 25% each.

^h Oral formula feedings with vitamin and mineral supplements (12, 13). Study subject 3(a) received 40% daily caloric intake as corn oil, 15% and 45% as protein and carbohydrate, respectively. Study subjects 3(b) and 5 received 35% daily caloric intake as cottonseed oil, 15% and 50% as protein and carbohydrate, respectively.

separated by TLC on silica gel G using a solvent system of isoctane-ethyl acetate-glacial acetic acid-*N*-butanol 70:35:10.5:10.5 (v:v:v:v); with this solvent system the critical separation of chenodeoxycholic acid from deoxycholic acid methyl ester was verified by GLC to be complete. Cholic and chenodeoxycholic acid methyl esters were isolated following visualization with I₂ vapor. The respective primary bile acid methyl esters were scraped into glass-sintered funnels and eluted with methanol. The eluate was divided volumetrically into two aliquots. In one, radioactivity was measured in a Packard Tri-Carb scintillation counter (Model 3380-3390, Packard Instrument Co., Inc., Downers Grove, IL) using an automatic activity analyzer (AAA Packard Instruments, Model 544) for quench corrections as previously described (2), with aquasol (New England Nuclear Corp., Boston, MA) as the scintillant. With the second aliquot, mass measurements were made by GLC after preparation of the trimethylsilyl (TMS) ether derivatives, using methyl hyodeoxycholate (Steraloids, Inc., Wilton, NH) as a quantitative standard. A 1% Hi-Eff 8 BP column (5 ft., 3 mm I.D.) (Applied Science Laboratories Inc., State College, PA) was employed for which the optimum conditions (2900 theoretical plates at chenodeoxycholic acid) were injector port 275°C, oven 225°C, detector 275°C, carrier gas N₂-flow rate 20 ml/min. Confirmation of the complete separation of chenodeoxycholic and deoxycholic acid TMS ethers was obtained in every case.

Fecal bile acid analysis

Fecal bile acid excretion was quantitated by the method of Grundy, Ahrens, and Miettinen (2). Stools were collected in 1-, 2-, or 4-day pools, so as to give a representative figure of bile acid excretion over a period of days ranging from 8 to 50, mean 20 days. Chromic oxide was used as a marker for correction of day-to-day variations in the fecal flow of acidic steroids (15); recovery exceeded 80% and was quantitatively similar to sitosterol recovery in all cases. 3 α , 7 α , Dihydroxy-12-keto-cholanic acid (Steraloids Inc., Wilton, NH) was used as an overall recovery standard, and quantification of total fecal bile acid mass as the respective TMS ether derivatives was carried out by GLC on a 1% SE-54 column (5 ft., 3 mm I.D.) (Applied Science Laboratories, Inc., State College, PA) using 5 α -cholestane as a quantitative standard. The optimal conditions found for this stationary phase were: injector port 265°C, oven 225°C, detector 265°C, carrier gas N₂, flow rate 40 ml/min. On this column the bile acid TMS ethers were poorly separated from each other, but well separated from materials other than bile acids. The retention time of the recovery

TABLE 2. Fecal bile acid quantitation by GLC on SE-54 columns, compared to data obtained on high-resolution columns (Hi Eff 8BP)

Patient	SE-54		HiEff 8BP		Percent Difference ^a
	mg/day	mmol/day	mg/day	mmol/day	
11	1376	3.44	1156	2.89	-19.0
12	392	0.98	352	0.88	-11.4
13	2092	5.23	2128	5.32	+1.7
14	436	1.09	404	1.01	-7.9
15	2344	5.86	2392	5.98	+2.0
16	556	1.39	620	1.55	+10.3
17	712	1.78	596	1.49	-19.5
Mean ^b					-3.5

$$^a \left(\frac{\text{HiEff 8BP minus SE-54}}{\text{HiEff 8BP}} \right) \times 100.$$

^b By paired *t*-test the differences were not significant ($P < 0.5$).

standard was longer than that of any of the naturally occurring bile acids in man, and thus mass was quantified by integrating the combined areas from the first peak (5 α cholestane) to the last (3 α , 7 α dihydroxy-12-ketocholanic acid), as previously described (2).

As evidence for the validity of bile acid quantification on SE-54 columns, seven separate stool samples from patients with widely different daily bile acid excretions (range 0.88 to 5.98 mmol/day) were subjected to GLC analysis on both 1% SE-54 and 1% Hi-Eff 8BP columns (Table 2). The seven subjects whose fecal bile acids were so examined represent a mixed group of hyperlipidemic phenotypes; patients 11, 13, 15, and 17 were on liquid formula feeding regimens, and patients 12, 14, and 16 were on solid food diets; patients 11, 13, and 15 were receiving cholestyramine 16 g/day for the period of their study. No statistical difference was found between the two sets of values. We conclude that the SE-54 column reliably estimates total fecal acid steroid mass (but not the mass of individual bile acids) and that, by virtue of the separation techniques prior to GLC, the data reported herein refer exclusively to fecal bile acids. Identification of individual peaks was possible on HiEff 8BP columns but not on SE-54 columns: more than 85% of the mass of fecal bile acid was accounted for by a mixture of deoxycholic, isodeoxycholic, and lithocholic acids in all patients.

These comparisons of GLC methodologies made it clear that our quantitation of total bile acids GLC on SE-54 columns was dependable, whereas for specific activity measurements of individual bile acids it was necessary to measure mass by GLC on HiEff 8BP columns. In reporting the results presented below, we have taken these differences in methodology into consideration.

Bile acid pool size, turnover, and daily synthesis rates were calculated independently for the two primary bile acids according to the method proposed by Lindstedt (1). The specific activity decay curves for each bile acid were analyzed by a standard least-squares fit program using an XDS Sigma 7 computer. Correlation coefficients for cholic acid ranged from 0.981 to 0.999 ($P < 0.05$). The mean standard errors for the two fractional turnover rates were 3.1% and 3.2%, respectively.

In 10 of 13 studies the correlation coefficient for both regression lines was maximal when the 24-hr specific activity value was included. In three studies (studies 1(b), 2, and 4) the 24-hr specific activity values were anomalous; hence, to obtain regression lines with the highest correlation coefficient, these early time points were ignored, and the regression line for both primary bile acids was calculated using specific activity points from 48 to 120 hr.

In eight studies where five to six time points were examined within the first 24 hr after infusion, the kinetic parameters were calculated by input-output analysis, the theoretical basis for which is presented elsewhere (16).

Statistical analysis

All statistical analysis was carried out by means of a Hewlett-Packard 97 calculator and the Student *t*-test program for paired and unpaired means supplied in the Hewlett-Packard Stat Pac I.

Characterization of bile acid specific activity curves within first 24 hr

Previous studies of the turnover of primary bile acids in man indicate that first-order kinetics apply and that pool sizes can be deduced by linear extrapolation of the specific activity decay curve back to time zero, yielding a theoretically maximum specific activity at time zero following instantaneous mixing within a pool of constant size. In eight studies in which bile was sampled at 4-hr intervals for the first 24 hr, only one subject (Patient 5) apparently displayed instantaneous mixing and ideal first-order kinetics (Fig. 1). In this subject, maximal specific activity for both primary bile acids was found at 4–8 hr, followed by linear decline thereafter with correlation coefficients of 0.998 and 0.999 for cholic and chenodeoxycholic acids, respectively.

The results from the other seven studies suggest that mixing of the label within the pool of primary bile acids occurs at different rates in different subjects. Those patients who were maintained on liquid formula feeding throughout the study demonstrated rapid mixing (Studies 3a, 3b, and 5), as evidenced by the fact that bile acid specific activity was maximal by 4 hr. These patients were fed five times daily, and it is likely that their bile acid pools cycled more rapidly than in the other five studies where solid food diets were consumed three times per day (Studies 1a, 1b, 1c, 2, 4). Correspondingly in the latter patients, complete mixing of the labeled bile acid within its pool took

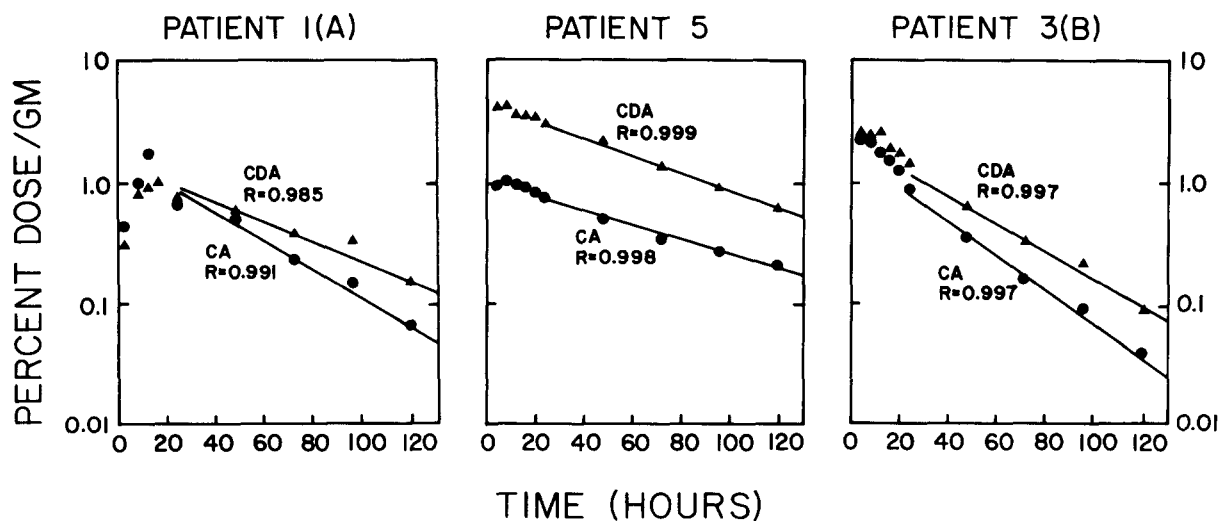


Fig. 1. Graphic representation of bile acid specific activity decay versus time. Note that the doses of labeled bile acids are normalized by expressing their radioactivities as percent dose/g bile acid. Three representative studies are shown in which [¹⁴C]cholic acid (CA) (●) and [¹⁴C]chenodeoxycholic acid (CDA) (▲) specific activity measurements were made five to six times within the first 24 hr following radio-labeled bile acid infusion. Regression lines were obtained by a least squares fit; correlation coefficients are shown adjacent to the regression line for the respective bile acid.

longer (range 12–42 hr). Various specific activity decay curves are shown in Fig. 1 to illustrate these points.

In Study 3(b) (Fig. 1), despite the fact that the peak specific activity was achieved 4 hr after infusion for both primary bile acids, the decay curve did not become log-linear until 24 hr, indicating that first-order kinetics did not apply for the first 24 hr after labeling of the bile acid pool. Similarly, in Studies 1(a), 1(b), 1(c), 3(a), and 4, log-linear specific activity decay curves were not achieved until 12–42 hr after the attainment of maximal specific activity. Our data are too few, however, to allow us to postulate the presence or absence of more than one component in a given curve.

In the eight studies in which data from early time points within the first 24 hr were subjected to input-output analysis, values for bile acid synthesis rates are shown in **Table 3**, and compared to the values obtained in the same study using conventional Lindstedt kinetics. On average there was no statistically significant difference in any parameter between the two methods, suggesting that input-output analysis offers no advantages over conventional isotope dilution kinetics for the determination of bile acid synthesis in man. Considered on an individual basis, however, there were some striking discrepancies, but no systematic trends were noted in comparing the two sets of calculations. For instance, in Patient 4 the synthesis rate by input-output analysis was only half that ob-

tained by Lindstedt kinetics for cholic acid, while for chenodeoxycholic acid the rates were fairly comparable. These results indicate that for cholic acid the area under the entire decay curve was much larger when the early time points were considered than when they were overlooked, indicative, we propose, of marked differences in mixing rates of the primary bile acids from patient to patient.

A comparison of bile acid production rates obtained by the two methodologies

The results of calculations based on the Lindstedt method (1) are given in **Table 4**. Differences in pool sizes, fractional turnover rates, and synthesis rates were analyzed according to hyperlipidemic phenotype but the differences did not approach statistical significance. Nevertheless, a trend could be observed toward higher bile acid production in hyperglyceridemic and mixed hyperlipidemics ($27.458 \pm 19.04 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) compared to hypercholesterolemic patients ($14.736 \pm 5.83 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) ($P > 0.01$). This observation was accounted for in large part by a more than twofold elevation in cholic acid synthesis in those patients with hyperglyceridemia.

For comparison, measurements of fecal bile acid output are presented in **Table 5**. In contrast to the results obtained by isotope dilution kinetics, chemical balance methodology yielded values for bile acid excretion that were quantitatively closer to one another for the two groups, with hypercholesterolemics

TABLE 3. Comparison of daily bile acid synthesis quantitated by conventional isotope dilution kinetics (Lindstedt method) and by input-output analysis in eight studies

Patient Phenotype ^a	Study	Daily Bile Acid Synthesis ^b							
		Lindstedt Method				Input-Output Analysis ^c			
		Cholic	Chenodeoxycholic	Total ^b		Cholic	Chenodeoxycholic	Total ^b	
		(mmol/day)	(mmol/day)	($\mu\text{mol}/\text{k} \cdot \text{day}$)	(mmol/day)	(mmol/day)	(mmol/day)	($\mu\text{mol}/\text{k} \cdot \text{day}$)	
1 HC	a	1.012	0.809	1.821	20.693	1.073	0.904	1.977	22.466
	b	0.599	0.680	1.279	14.872	0.810	0.836	1.646	19.140
	c	1.327	0.459	1.786	20.889	1.113	0.451	1.564	18.292
2 HC		0.303	0.315	0.618	7.630	0.354	0.358	0.712	8.790
3 MH	a	2.036	0.952	2.988	36.439	1.681	0.857	2.538	30.951
	b	1.080	0.648	1.728	23.357	0.944	0.617	1.561	21.095
4 MH		3.830	0.919	4.749	65.958	1.658	0.789	2.447	33.986
5 HG		0.923	0.213	1.136	16.955	0.981	0.224	1.205	17.985
Mean \pm SD		1.389 \pm 1.11	0.624 \pm 0.27	2.013 \pm 1.30	25.848 \pm 18.17	1.077 \pm 0.44	0.630 \pm 0.26	1.706 \pm 0.61	21.588 \pm 7.89

^a Abbreviations: HC, hypercholesterolemia; MH, mixed hyperlipidemia; HG, hyperglyceridemia.

^b Results for individual bile acid synthesis rates are expressed as mmol per day. Total synthesis rates expressed as mmol per day and μmol per kilo body weight per day.

^c Synthesis rates derived from the general equations for input-output analysis (16).

TABLE 4. Daily bile acid synthesis by conventional isotope dilution kinetics (Lindsredt method)

Patient ^a Phenotype	Study	Cholic Acid						Chenodeoxycholic Acid					
		Pool (nmol)	Synthesis (nmol/day)	FTR ^b	Synthesis μmol/k·day	Pool (nmol)	Synthesis nmol/day	FTR	Synthesis μmol/k·day	mmol/day	Total Bile Acid Synthesis μmol/k·day	mg/kg/day	
1 HC	a	1.621	1.012	0.624	11.494	2.146	0.809	0.377	9.185	1.821	20.693	8.277	
	b	0.985	0.599	0.608	6.965	1.585	0.680	0.428	7.907	1.279	14.872	5.949	
	c	3.367	1.327	0.394	14.488	3.301	0.459	0.139	5.357	1.786	20.889	8.356	
2 HC		0.998	0.303	0.304	3.741	1.128	0.315	0.279	3.889	0.618	7.630	3.052	
3 MH	a	2.330	2.036	0.874	24.848	1.363	0.952	0.698	11.616	2.988	36.439	14.576	
	b	1.395	1.080	0.774	14.595	0.975	0.648	0.665	8.784	1.728	23.351	9.340	
4 MH		7.952	3.830	0.481	53.194	2.496	0.919	0.369	12.764	4.749	65.958	26.383	
5 HG		1.900	0.923	0.486	13.769	0.493	0.213	0.432	3.172	1.136	16.955	6.782	
6 MH		0.330	0.536	1.623	10.113	0.706	0.550	0.779	10.330	1.086	20.491	8.196	
7 HC		0.530	0.722	1.363	8.639	0.818	0.635	0.776	7.593	1.357	16.232	6.493	
8 HC		1.218	0.256	0.210	4.227	1.905	0.234	0.123	3.872	0.490	8.099	3.240	
9 MH		0.353	0.288	0.815	3.885	0.583	0.262	0.450	3.547	0.550	7.432	2.973	
10 MH		1.618	1.330	0.822	14.778	1.110	0.612	0.551	6.797	1.942	21.578	8.631	
Mean HC (±SD) (n = 6)		1.453 ± 1.00	0.703 ± 0.41	0.584 ± 0.42	8.214 ± 4.21	1.814 ± 0.88	0.522 ± 0.22	0.354 ± 0.24	6.301 ± 2.24	1.225 ± 0.57	14.736 ± 5.83	5.895 ± 2.33	
Mean MH + HG (±SD) (n = 7)		2.268 ± 2.62	1.432 ± 1.20	0.839 ± 0.38	19.312 ± 16.20	1.104 ± 0.69	0.594 ± 0.29	0.563 ± 0.15	8.144 ± 3.79	2.026 ± 1.43	27.458 ± 19.04	10.983 ± 7.61	

^a HC, hypercholesterolemia; MH, mixed hyperlipidemia; HG, hyperglyceridemia.

^b FTR, fractional turnover rate in %/day.

TABLE 5. Daily bile acid synthesis data in 13 experiments

Patient ^a Phenotype	Study	Pools Analyzed ^b	By Chemical Analysis				By Isotope Dilution
			Mean Daily Bile Acid Excretion ^c				
			mmol/day	mg/day	μmol/kg·day	mg/kg/day	
1 HC	a	10 (1-2)	2.105 ± 1.578	842 ± 631	23.921	9.568	8.277
	b	9 (1)	1.273 ± 0.380	509 ± 152	14.797	5.919	5.949
	c	8 (1)	2.035 ± 1.512	814 ± 607	23.801	9.520	8.356
2 HC		11 (1)	0.618 ± 0.110	247 ± 44	7.624	3.050	3.052
3 MH	a	12 (2)	0.865 ± 0.138	346 ± 55	10.549	4.220	14.576
	b	10 (2-4)	1.323 ± 0.650	529 ± 260	17.872	7.149	9.340
4 MH		5 (4)	0.585 ± 0.113	234 ± 45	8.125	3.250	26.383
5 HG		11 (1-4)	0.835 ± 0.145	334 ± 58	12.463	4.985	6.782
6 MH		12 (1)	0.830 ± 0.453	332 ± 181	15.660	6.264	8.196
7 HC		8 (1-4)	1.093 ± 0.525	437 ± 210	13.068	5.227	6.493
8 HC		27 (1-4)	0.495 ± 0.260	198 ± 104	8.182	3.273	3.240
9 MH		9 (1-4)	0.873 ± 0.338	349 ± 135	11.791	4.716	2.973
10 MH		9 (1-4)	0.850 ± 0.190	340 ± 76	9.444	3.778	8.631
Mean HC (±SD) (n = 6)			1.270 ± 0.68	508 ± 274	15.232 ± 7.23	6.093 ± 2.89	5.895 ± 2.33
Mean MH + HG (±SD) (n = 7)			0.880 ± 0.22	352 ± 88	12.272 ± 3.45	4.909 ± 1.38	10.983 ± 7.61

^a HC, hypercholesterolemia; MH, mixed hyperlipidemia; HG, hyperglyceridemia.

^b Total number of pools analyzed (figures in parenthesis indicate 1-, 2-, 3-, or 4-day collections).

^c Mean ± S.D.

(15.232 ± 7.23 μmol·kg⁻¹·day⁻¹) excreting slightly more bile acids than hyperglyceridemics (12.272 ± 3.45 μmol·kg⁻¹·day⁻¹); however, these differences were not statistically significant.

Correlations between the two different methods are

shown graphically in Fig. 2. When the results of all 13 studies were compared, the correlation coefficient was close to zero (Fig. 2A). However, by separating normoglyceridemic from hyperglyceridemic individuals and plotting separate regression lines for each

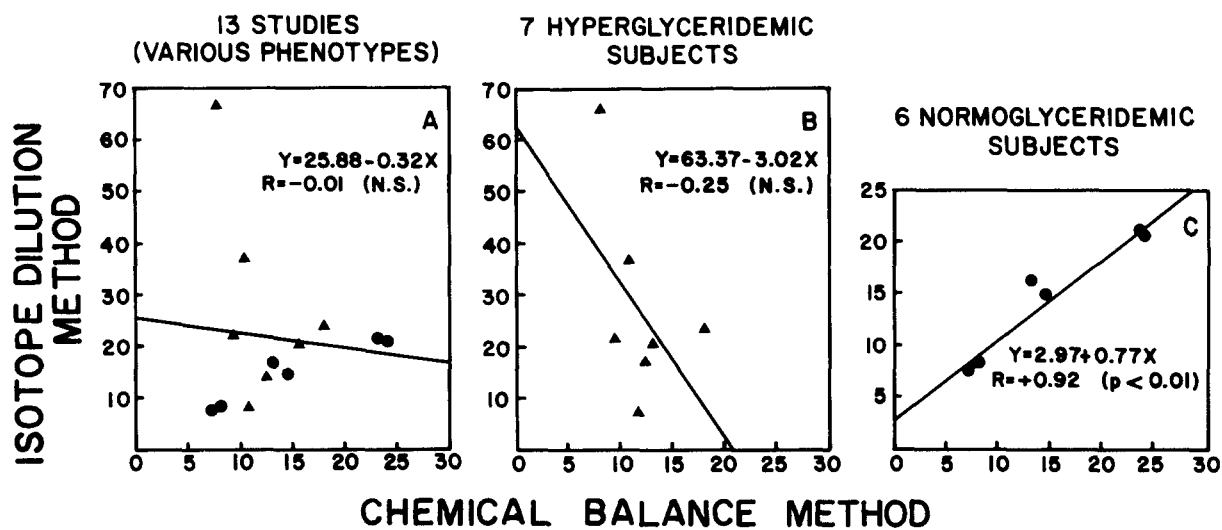


Fig. 2. Daily rates of bile acid synthesis in hyperlipidemic subjects. Graphic representation of total bile acid synthesis rates evaluated in 13 patients by isotope dilution (vertical axis) and chemical balance methodology (horizontal axis). All data are expressed as μmol·kg⁻¹·day⁻¹. Closed circles represent hypercholesterolemic patients; closed triangles represent hyperglyceridemic patients.

group, it can be seen that, for those subjects without elevated plasma triglycerides (Fig. 2C), the correlation between the data obtained by the Lindstedt method (1) and chemical balance methodology is positive and high (+0.92, $P < 0.01$), whereas for hyperglyceridemic individuals (Fig. 2B) the correlation is negative and weak (-0.25, NS). It should be stressed that the number of stool pools analyzed and whether they represented 1, 2, or 4 days' collection had no independent effect on the level of agreement between the two methodologies.

Fig. 2 suggests that the Lindstedt method is appropriate for studies of bile acid synthesis in normoglyceridemic patients by virtue of the strong correlation between the data obtained by the two methods. However, in hyperglyceridemic patients, the results obtained by the two methods are completely at variance, with no apparent correlations found in any of the seven studies. According to the chemical balance method, the daily synthesis rates of total bile acids in these seven studies varied from 8.1 to 17.9 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, whereas the Lindstedt method yielded results ranging from 7.4 to 66 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$; the % differences between the two sets of data varied from -37% to +712%, mean +163%.

DISCUSSION

These results show that simultaneous measurements of bile acid production in man by isotope dilution kinetics and chemical balance methodology correlate well in hypercholesterolemic individuals but poorly in subjects with elevated plasma triglycerides. Differences in bile acid production have been previously correlated with disturbances in triglyceride metabolism (3-7, 17, 18), but such studies have utilized either isotope dilution kinetics (3-7) or chemical balance methodology (19, 20), never the two together. In addition to the possible role of hyperlipidemic phenotype in these observed differences in bile acid metabolism, there is dispute as to whether or not the isotope dilution method itself leads to overestimates of bile acid production as compared to chemical balance methodology in the same patient (8-11). The strengths and weaknesses of each method are clearly of central importance in any systematic comparison of methodologies under differing conditions. The basic prerequisites for study by the isotope dilution method have been extensively reviewed (21); they include *i*) constant bile acid pool size; *ii*) complete mixing of the labeled bile acids within their respective pools (22); *iii*) no isotope effects in the conjugation of bile acids (23); *iv*) no reentry of label into the pool from

which it has been removed—for example, by rehydroxylation of deoxycholic acid, as in the rat (24); and *v*) a truly single exponential decay of specific activity (an unrecognized second exponential appearing after sampling has ended would lead to an overestimate of pool size and hence synthesis rates).

The critical prerequisites for a valid measurement of fecal bile acid excretion in man have been outlined in detail elsewhere (2). The protocol involves maintenance of clinical steady-state conditions (stable body weight, constant caloric and steroid intakes, and reliable recovery (>80%) of chromic oxide as a fecal flow monitor), the use of an internal standard to correct for procedural losses, separation and characterization of bile acids first as the methyl ester derivatives on TLC and subsequently as the trimethylsilyl ether derivatives by GLC.

Proof that the final fecal bile acid fraction accounts for all the fecal bile acids and nothing but bile acids has previously been reported (2). In the present report we show that quantitation of total fecal bile acid mass by GLC using a 1% SE-54 column is accurate to within 5% of the value obtained with a high resolution column packing (1% HiEff 8BP). We thus feel confident that our measurement of total fecal bile acids by this methodology is valid and does not lead to systematic overestimates.

Since fecal bile acid measurements are made continuously in 1- to 4-day pools over at least a 2-week period, the several results obtained in any single study, on being averaged, demonstrate a considerable variability that we have considered to be mainly physiological in origin, rather than methodological. From an interpretative viewpoint the demonstration of this variability has its advantages, but from a statistical standpoint the large standard deviations often work against the possibility of demonstrating significant differences between results obtained in various patients or patient-groups. While this fact necessarily weakens comparisons between subjects based on results of chemical balance measurements, it does not necessarily affect comparisons between results obtained by chemical balance methods and isotope dilution kinetics in the *same* subject or group of subjects.

Differences attributed to hyperlipidemic phenotype

Using isotope dilution kinetics, studies by Einarsson and Hellström (3) and Kottke (4) indicated that bile acid synthesis was greater in patients with hyperglyceridemia than in hypercholesterolemic subjects. In both of these studies investigators relied on the use of randomly tritiated chenodeoxycholic acid and [^{14}C]cholic acid; the former has been shown to be

accompanied by variable degrees of chemical and biological exchangeability of tritium (25, 26), and its use might be expected to yield an overestimate of bile acid production. There were reproducible differences, however, between hyperglyceridemics and hypercholesterolemics tested with the same labeled bile acids, and values for bile acid production for the hypercholesterolemic group were within the range for similar subjects using [24-¹⁴C]cholic and [24-¹⁴C]chenodeoxycholic acid. Thus four hypercholesterolemics studied by Einarsson and Hellström (3) yielded a mean value of 12.21 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, and for ten hypercholesterolemics studied by Kottke (4), the mean value was 12.07 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$. These figures are within the range of our own results (14.74 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) and those of other investigators for hypercholesterolemic subjects (Angelin et al. (5) 10.70 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, and Einarsson et al. (6) 15.18 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$), using only ¹⁴C-labeled bile acids.

In contrast to the values obtained in hypercholesterolemics, the use of randomly tritiated chenodeoxycholic acid yielded values for bile acid production in hyperglyceridemics that appear disproportionately elevated. Thus, Einarsson and Hellström (3) and Kottke (4) reported mean values for hyperglyceridemics of 45.47 and 43.07 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, respectively, whereas investigators using ¹⁴C-labeled bile acids (5, 6) report values in the range of 20 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$.

Thus, despite an apparent overestimate of the absolute rates of bile acid synthesis consequent upon the use of biologically unreliable [³H]chenodeoxycholic acid, both investigators demonstrated a clearly elevated (relative) rate of bile acid production in hyperglyceridemic patients.

Bile acid production in man quantitated by chemical balance methodology

For reasons cited earlier, few investigators have systematically compared fecal bile acid excretion in hypercholesterolemic and in hyperglyceridemic individuals. In one study Grundy et al. (20) compared a group of seven normoglyceridemic and thirteen hyperglyceridemic individuals; the former group consisted of two normolipidemics and five hypercholesterolemics, the latter group, a mixture of Fredrickson types I, III, IV, and V. Fecal bile acid excretions for the two groups, considered as representative of normoglyceridemic and hyperglyceridemic subjects, were 9.42 and 12.71 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, respectively; these values were not statistically different from one another.

Detailed inspection of the literature deriving from our own (20, 27, 28) and other laboratories using

similar methodology (19, 29–31) indicates that bile acid excretion in man averages 11 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ (10.76 \pm 2.95 SD). This figure is derived from seven studies involving 83 subjects—normals, hyperglyceridemics and hypercholesterolemics of all Frederickson phenotypes, including homozygous and heterozygous familial hypercholesterolemia (29, 30), on various dietary regimens of saturated and polyunsaturated fats (19, 28, 31). Our conclusion from this literature search is that, when measurements are made by chemical balance methodology, there are no systematic differences in bile acid excretion between hypercholesterolemic and hyperglyceridemic patients.

Comparisons of two methodologies in the same patient

There are four reports in the literature that compare the two methodologies for measurement of bile acid production in the same patient. We have chosen to exclude two of these reports from this discussion: that of Miller and Nestel (10) who reported only two patient-studies, and that of Vlahcevic, Gregory, and Swell (11) whose chemical balance studies were carried out without the administration of markers to correct for day-to-day variations in fecal flow. However, the reports of Tangedahl, Hofmann, and Kottke (9) and Subbiah et al. (8) are relevant and merit discussion in some detail.

Tangedahl et al. (9), reporting a study of four hypercholesterolemics, found discrepancies in bile acid production as quantitated by isotope dilution kinetics versus chemical balance techniques; in all cases, isotope dilution kinetics gave a higher value (range 8.2% to 247%) than chemical balance methodology. Their values for bile acid excretion by chemical balance methodology were within one standard deviation of the value derived from our literature survey; however, their values for bile acid synthesis by isotope dilution kinetics were higher than results obtained in hypercholesterolemic subjects by other workers.

In their study Tangedahl et al. (9) carried out isotope dilution kinetic studies using [11,12-³H]chenodeoxycholic and [24-¹⁴C]cholic acid. The former was shown (32) to lose between 10 and 20% of the administered dose by biological ³H exchange with a mean of 13%, so Tangedahl et al. (9) multiplied their raw data by 1.13. In studies carried out with randomly tritiated chenodeoxycholic acid, Einarsson, Hellström, and Kallnen (25) demonstrated biological ³H loss to be of the order of 17%. Earlier, Panveliwala, Pertsemliadis, and Ahrens (26) reported that randomly tritiated chenodeoxycholic acid, tested in

one patient against [24-¹⁴C]chenodeoxycholic acid, was subject to 55% ³H loss by biological exchange. These reports of heterogeneity in the degree of ³H loss, observed with different batches of randomly tritiated bile acids, are reminiscent of the variable unreliability we have reported in batch-to-batch comparisons of tritiated cholesterol (33).

Concern over the variability of losses due to tritium exchange is also germane to our evaluation of the study of Subbiah et al. (8); they studied nine patients, a heterogeneous group of hypercholesterolemics, mixed hyperlipidemics, hyperglyceridemics, and one normal volunteer. Subbiah et al. (8) reported that chemical balance methodology gave lower results than isotope dilution kinetics in all cases (18.1 to 44.2%); in six of their subjects, primary bile acid kinetics were studied with the use of randomly tritiated chenodeoxycholic acid and [24-¹⁴C]cholic acid. However, one subject was studied with randomly tritiated and, at a later date, [24-¹⁴C]chenodeoxycholic acid; [24-¹⁴C]cholic acid was co-administered on both occasions. Bile acid synthesis using the randomly tritiated chenodeoxycholic acid was 28% higher than that obtained with [24-¹⁴C]chenodeoxycholic acid. We have applied the figure of 28% ³H loss found in this one patient as a general correction factor for all six patients studied with [³H]chenodeoxycholic acid; we found no statistical difference between the values for bile acid production obtained by isotope dilution kinetics and chemical balance methodology (9.262 ± 4.22 and 10.320 ± 3.28 μmol·kg⁻¹·day⁻¹, respectively). Subbiah's three other patients were given 24-¹⁴C-labeled bile acids only; they were all hyperglyceridemic (two type III, one type V) and all had higher bile acid synthesis values by isotope dilution kinetics than by chemical balance methodology, 20.467 ± 23.49 and 8.187 ± 9.39 μmol·kg⁻¹·day⁻¹, respectively.

In comparing our present results with those discussed above, we are led to the conclusion that isotope dilution kinetics may give a value for bile acid synthesis in hyperglyceridemic states that is clearly different from the results obtained by chemical balance methodology. At least some of the discrepancies can be attributed to the use of biologically unreliable tritiated bile acids. On the other hand, the differences that are persistently found in hyperglyceridemic states may reflect a fundamental hepatocellular disturbance of bile acid metabolism that is unique to this disease. ■

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