Within-day Fluctuations in Serum Bile-acid Concentrations among Normal Control Subjects and Patients with Hepatic Disease

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Engelking, Larry R., Dasher, Charles A., and Hirschowitz, Basil I.: Within-day fluctuations in serum bile-acid concentrations among normal control subjects and patients with hepatic disease. Am J Clin Pathol 73: 196–201, 1980. Under carefully controlled conditions in a clinical research ward, conjugated primary bile-acid concentrations were measured by a ^{125}I-radioimmunoassay every eight hours for four days in seven healthy control subjects and ten patients with hepatic disease (five with cirrhosis, three with primary biliary cirrhosis and two with sclerosing cholangitis). Two meals of approximately equal composition were consumed daily at 10 A.M. and 6 P.M., and blood was drawn at 4 A.M., 12 noon and 8 P.M.. With the exception of one patient, all subjects had greater postprandial than fasting serum bile-acid concentrations, with all healthy control subjects and most of those with hepatic disease showing evening values equal to or greater than the noon values. For the healthy control subjects, the mean values were 0.8, 1.4 and 1.9 μM, and for those with hepatic disease, 108, 140 and 133 μM. There were large fluctuations in serum bile acids (up to sevenfold) among patients with hepatic disease. These fluctuations were independently validated by finding corresponding changes in serum radioactivity derived from injection of a tracer (24-^{14}C cholic acid) at the start of each study.

To be consistent, especially for serial measurements, bile acids should be measured in blood taken at the same time of the day and at the same time relative to meals. (Key words: Radiolabelled cholic acid; Fasting serum bile acid; Postprandial bile acid; Cirrhosis; Cholestasis; ^{125}I-radioimmunoassay.)

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THE CONCENTRATION of bile acids in the peripheral circulation fluctuates during the day,[13,14] and at any one time depends on a balance between the load of bile acids presented to the liver and the capacity of the liver to extract it from the blood. The load of bile acids presented to the liver for uptake is related to the load on the intestine, *i.e.*, gallbladder contraction after meals,[3,17] on the capacity of the bowel to absorb bile acids,[20] on blood flow to the liver,[22] which may be disrupted by portal systemic shunting,[18] and on the rates of bile-acid[5,21] and cholesterol synthesis.[1] The rate of hepatic clearance of bile acids in control subjects is independent of the load.[19] It is impaired in hepatobiliary disease, in which there could be defects of uptake, conjugation, or excretion,[11] mechanisms which could also be influenced by various drugs.[7,16] The capacity of the healthy liver to extract and excrete bile acids exceeds the load imposed upon it by intestinal absorption into the portal circulation, so that the systemic blood levels remain low and fluctuate within relatively narrow limits.

During the course of a study of healthy control subjects and patients with hepatic disease, the serum concentration of bile acids was determined three times a day for four days.[8] Large fluctuations in serum bile acid concentrations were found. This report examines those fluctuations in relation to the two meals a day the subjects were allowed, and with respect to the type of hepatic disease. The patients were selected for study to represent two broad types of hepatic disease—those with pronounced cholestasis which included advanced primary-biliary cirrhosis and sclerosing cholangitis, and those with stable cirrhosis in whom cholestasis was not predominant (as shown by total bilirubin values ≤27 μM or 1.6 mg/dl).

Materials and Methods

Seventeen subjects were studied after written, informed consent was obtained. The participants consisted of seven control subjects who had neither clinical nor laboratory evidence of hepatobiliary disease, and ten patients with hepatic disease documented by liver biopsy and appropriate laboratory tests. Five had well-established but stable cirrhosis and five had advanced cholestasis (three with primary-biliary cirrhosis, two with sclerosing cholangitis) (Table 1). Cholestyramine and neomycin therapies, if being given (Table 1), were stopped at least four days before study. The subjects were hospitalized for four days in a clinical research unit during the course of the study. They were allowed two complete solid meals (each about 1,000 calories),

Received December 11, 1978; received revised manuscript and accepted for publication February 14, 1979.

Supported in part by the Division of Gastroenterology and NIH Clinical Research Grant RR32.

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0002-9173/80/0200/0196 $00.80

the first at approximately 10 A.M., consisting of eggs, meat, toast with butter, and milk, tea or coffee, and the second at approximately 6 P.M., a normal, full, hospital supper of meat, vegetables, dessert and beverage. Blood samples were drawn two hours after each meal, as well as at about 4–5 A.M., succeeding samples separated by an eight-hour interval. Except for water, in-between-meal beverages or snacks were not allowed.

Ten–50 μCi of 24-^{14}C-cholic acid (SA 46.7 μCi/μM, sterile pyrogen-free, New England Nuclear) were injected into an antecubital vein eight hours prior to the start of study. Isotope concentrations were used for an independent validation of changes in serum bile-acid concentrations. Liquid scintillation counting of serum (0.5 ml) was performed in 10-ml Beckman Ready-Solv H.P. using external standardization on a Packard Tri-Carb scintillation spectrometer. Results were expressed as a percentage of the injected dose per liter of serum after correcting for biological ^{14}C-bile acid turnover with respect to time.[8,9] Serum bile-acid concentrations were determined by an ^{125}I-radioimmunoassay as previously described,[23] with the antibody used which has the following cross-reactivity (taurocholic acid 100, glycocholic acid 36, cholic acid 10, glychochenodeoxycholic acid 36, taurochenodeoxycholic acid 1). A taurocholate standard gave values for serum bile-acid concentrations,[10] which correlated well with the 3-α OH steroid dehydrogenase method of Barnes and Chitranukroh[2] for total bile acids over the range at 1 to 300 μM (^{125}I-radioimmunoassay = 1.15* total bile acids + 3.8, r = +.844, n = 149, $P < 0.0001$). The ^{14}C-cholic acid metabolites were assumed to be uniformly distributed throughout the total bile-acid pool so that variations in serum radioactivity could be correlated with variations in serum bile-acid concentration. For the control subjects, the concentration of radioactivity was too small for this correlation to be made. Analysis of individual bile acids in bile by thin-layer chromatography from patients with hepatic disease demonstrated insignificant conversion of the radioactivity to ^{14}C-deoxycholic acid. Analysis of variance, Students' unpaired *t*-test, paired *t*-test, and least-squares regression analysis were used for data analysis.

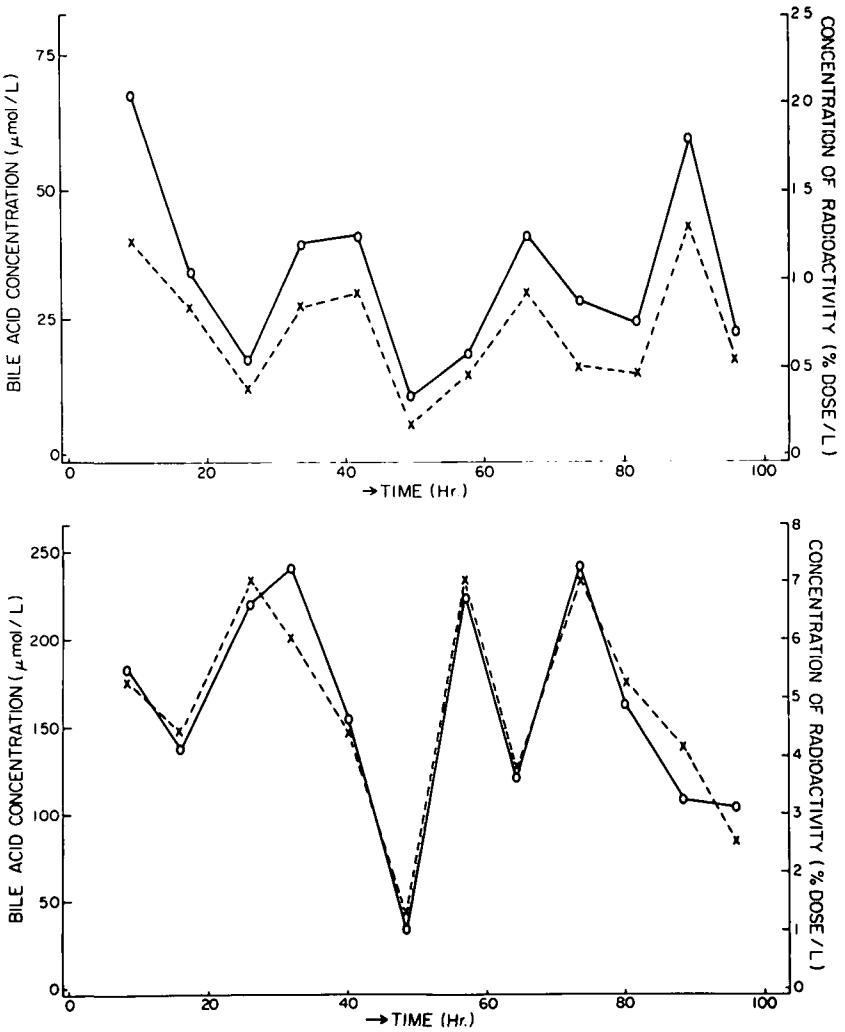

FIG. 1. Variations in serum bile acid concentrations determined by radioimmunoassay (x) and by liquid scintillation of 34-^{14}C-cholic acid (o). The upper curve represents values for Patient 10, and the lower curve values for Patient 12, both cirrhotic. Radioactivity measurements have been corrected for biological ^{14}C-bile acid turnover with respect to time.

Table 1. Clinical Data and Plasma Concentrations of 17 Subjects

	Age (Years), Sex	Diagnosis	Cholecystectomy	Varices	Medication
Patient 1	30, F	Normal	No	No	—
Patient 2	49, F	Normal	No	No	Estrogen
Patient 3	30, F	Normal	No	No	—
Patient 4	21, F	Normal	No	No	—
Patient 5	24, F	Normal	No	No	—
Patient 6	28, M	Normal	No	No	—
Patient 7	24, F	Normal	No	No	Estrogen
Patient 8	55, F	Postnecrotic cirrhosis	No	Shunted	Aldactone, neomycin
Patient 9	66, F	Methotrexate cirrhosis	No	Shunted	Aldactone, neomycin
Patient 10	66, F	Chronic active hepatitis with cirrhosis	No	No	Aldactone, azathioprine
Patient 11	58, F	Chronic active hepatitis with cirrhosis	No	No	Azathioprine, prednisone
Patient 12	72, M	Postnecrotic cirrhosis	No	Yes	Aldactone
Patient 13	49, F	Primary biliary cirrhosis	Yes	Yes	Phenobarbital, cholestyramine, 25-OH vitamin D
Patient 14	50, F	Primary biliary cirrhosis	Yes	Yes	Neomycin, phenobarbital, 25-OH vitamin D
Patient 15	37, F	Primary biliary cirrhosis	No	No	Azathioprine, cholestyramine
Patient 16	45, M	Sclerosing cholangitis	Yes	Yes	Cholestyramine
Patient 17	23, M	Sclerosing cholangitis	Yes, T-tube	No	—
	Normal Range				

Results

The mean peripheral venous serum bile-acid concentrations for each subject studied are listed in Table 2. Analysis of variance of each patient's serum bile-acid concentrations at each time indicated that the day-to-day variations were not significant ($P > |F| = 0.5–0.93$), yet there were several large changes in serum bile acid concentrations at different times during the day. In patients with hepatic disease, changes in serum concentration measured by the bile-acid radioimmunoassay corresponded to changes in serum ^{14}C-radioactivity. A gradual decay of specific radioactivity in the serum resulted from ^{14}C-bile-acid turnover, which was monoexponential. When corrected for this decay, changes observed in the serum concentration by the two methods were in close agreement. Data from two patients are illustrated in Figure 1.

With each subject serving as his own control, the paired *t*-test was used to detect concentration differences within each group (normals, cirrhosis, cholestasis) to obtain a reduced and better estimate of the variance, as opposed to an overall comparison of the means. Table 3 shows the within-group comparisons. Within the group of control subjects, each time of day appeared to be uniquely different, with the significant concentration gradient being postprandial evening > postprandial midday > fasting. Both the evening value (Table 3) and the average of the two postprandial samples were higher than the fasting value, both in controls ($P < 0.020$) and in the patients with hepatic disease ($P = 0.02$). When the patients with hepatic disease were examined in two subgroups, in those with cirrhosis the postprandial evening serum bile-acid concentrations were significantly greater than in the fasting state ($P < 0.05$). There were no significant differences between the postprandial values. In the cholestasis group the highest value occurred after the first meal of the day, but this change did not quite reach significance ($t = 2.375$, $df = 4$, $P > 0.05$). The evening value was lower than the noon value in all five, and lower than the fasting value in two.

Discussion

In four consecutive days of observation of each subject, the two-hour postprandial daytime serum bile-acid concentrations were consistently elevated above the early-morning fasting levels in all the normal subjects and in nine of the ten patients with hepatic disease. Previous studies have shown increased postprandial concentrations among patients with histologically proven hepatic disease,[6] but while some investigators have not found such increases in control subjects above their early-morning fasting levels,[3,11,17] others have reported postprandial elevations of serum bile acids in normal subjects measured both by radioimmunoassay[20] and by enzymatic analysis[10] following liquid meals. More recently, Hepner and Demers,[12] using multiple radioimmunoassays, have shown that following solid meals there is a more prolonged elevation of serum bile-acid concentrations. Our data confirm

(Biochemical Values Determined the First Day of the Study)

Serum Glutamic-oxalacetic Transaminase (IU/l)	Alkaline Phosphatase (IU/l)	Prothrombin Time (sec)	Albumin (g/dl)	γ-Globulin (g/dl)	Bilirubin (T) (μM)	Hematocrit (%)
20	54	0	4	0.7	5.1	36
12	62	0	4.2	1.2	5.1	38
15	52	0	4.1	0.8	5.1	42
28	66	1.5	3.9	0.9	8.5	39
30	74	0.8	4.1	1.0	10.2	44
26	70	0	4.0	1.8	5.1	35
20	42	0	4.2	0.9	8.5	43
43	134	2.4	2.9	2.5	27	44
26	196	0.4	3.0	2.4	12	32
101	86	1.0	3.0	0.9	19	35
103	160	1.1	3.3	2.2	27	41
110	274	1.8	3.2	1.5	20	46
380	680	0.7	3.3	2.0	60	36
121	998	5.7	3.5	1.3	265	30
114	388	0	4.3	1.1	46	40
181	680	1.6	3.0	1.8	272	29
157	1170	0	3.6	2.2	274	32
7–40	25–115		>3.5	0.6–1.5	0–20	34–44 female 39–49 male

that. In some of the patients with hepatic disease we found quite a wide fluctuation in serum bile acid concentrations within the day (as much as sevenfold in an eight-hour interval; see, *e.g.*, Fig. 1). Such data indicate that variations in sampling could explain many of the conflicting results described by others.

In those subjects with no hepatic disease and with an intact gallbladder, serum bile acids were almost doubled after the first meal of the day and more than doubled in the evening, as observed by Hepner and Demers.[12] In our patients with hepatic disease in whom fasting serum bile-acid concentrations were more than 100 times greater than normal, there was no difference between the two postprandial values, both being approximately 25% above fasting values.

The absolute increases in serum bile acids (13 to 123 μM) reflect the inability of the damaged liver to remove bile acids from the portal circulation due to either portosystemic shunting or impaired portobiliary transfer.[4,18] The number of subjects in each subgroup was too small to examine the role of the gallbladder, of shunting or varices, or of drugs which affect bile-acid metabolism, or even to clearly separate cholestasis from cirrhosis, though the former showed the greater bile-acid changes.

Because of wide fluctuations sometimes seen in serum bile-acid concentrations within the day, no one value is typical of a subject. However, the standard error for each individual in the fasting state was smaller than in the postprandial state. In the ten subjects with

Table 2. Individual Venous Blood Serum Bile-acid Concentrations*

	No. Days Studied	Fasting	Two-hour Postprandial	
			Midday	Evening
Normal				
Patient 1	4	.6 ± .3	1.5 ± 1	1.8 ± .2
Patient 2	4	.5 ± .1	.4 ± .2	1 ± .1
Patient 3	4	0.6 ± .1	.4 ± .1	1.7 ± .3
Patient 4	4	0.9 ± .1	1.1 ± .1	1.8 ± .7
Patient 5	4	.6 ± .1	3 ± .5	3.1 ± .4
Patient 6	4	0.8 ± .2	1.1 ± .4	1.5 ± .2
Patient 7	4	1.8 ± .5	2.3 ± .3	2.9 ± .5
Group Mean No. of Subjects	7	.8 ± .2	1.4 ± .4	1.9 ± .3
Cirrhosis				
Patient 8	4	149 ± 18	178 ± 68†	192 ± 15
Patient 9	3	43 ± 7	66 ± 9†	68 ± 24
Patient 10	4	138 ± 6	147 ± 50	196 ± 13
Patient 11	4	51 ± 9	59 ± 17	86 ± 16
Patient 12	4	27 ± 4	11 ± 2	22 ± 6
Group Mean No. of Subjects	5	82 ± 26	91 ± 31	113 ± 35
Cholestasis				
Patient 13	4	30 ± 3	44 ± 6	41 ± 6
Patient 14	4	233 ± 27	254 ± 29†	241 ± 22
Patient 15	4	87 ± 6	235 ± 62	152 ± 24
Patient 16	5	164 ± 4	213 ± 26†	160 ± 14
Patient 17	4	187 ± 4	204 ± 9	202 ± 17
Group Mean No. of Subjects	5	140 ± 36	190 ± 37	159 ± 33

* (μM, Means ± SEM). Each subject was studied for four days except as noted.
† One observation missing for the midday mean.

Table 3. Serum Bile-acid Concentrations

		Fasting		2 hr Postprandial			
		Morning (μM ± SE)	Δ*	Midday (μM ± SE)	Δ*	Evening (μM ± SE)	Δ* (Difference between Evening and Morning)
Normals	n = 7†	.78 ± .18		1.38 ± .26		1.96 ± .26	
	Δ		.60 ± .26		.58 ± .17		−1.18 ± .25
	P		.127		.016		.003
Hepatic disease	n = 10†	111.9 ± 23		141 ± 28		136 ± 2.4	
	Δ		30 ± 14		−5 ± 12		−25 ± 10
	P		.053		.73		.045
Cirrhosis	n = 5†	82 ± 26		91 ± 31		113 ± 35	
	Δ		10 ± 6.7		21 ± 8		−31 ± 10.5
	P		.304		.050		.045
Cholestasis	n = 5†	140 ± 36		190 ± 37		159 ± 33	
	Δ		50 ± 25		−31 ± 17		−19 ± 12
	P		.081		.130		.090

* Δ: mean differences ± SEM between adjacent columns.
† Each subject was studied for four days (see Table 2).

hepatic disease (Table 2) the mean standard error was 9.8% for fasting subjects (n = 10), and 17.8% for postprandial (n = 20). In turn, for the four cholesystectomized subjects, mean standard error for fasting samples was 6.5%, compared with 12% for the six patients with gallbladders. For the postprandial samples, the values are 10.3% and 22.8%, respectively. Thus, for long-term follow-up of a subject with hepatic disease, bile acids studied in the fasting state would be subject to less variation.

In the patients with hepatic disease we studied, serum bile acids in both fasting and postprandial states were many times greater than normal, but many patients with early hepatic disease have been reported to have normal fasting values,[3,17,24] though serum bile-acid elevation remains the one abnormality most commonly found in patients with hepatic disease.[6,15,16,19]

The number of subjects in this study is small, but they were studied under carefully-controlled conditions. What can be deduced from this study is that to derive consistent results for any one person, serum bile acids should be measured at the same time of day, and at the same time relative to meals. It is clear, however, that for the respective condition (fasting, postprandial), a serum bile-acid concentration elevated above the control range means that the liver does not adequately clear the bile acid presented to it for excretion, and this is a significant dysfunction. No diagnostic or pathophysiologic conclusions can yet be drawn from the pattern of fluctuation within any one day or in any one subject.

Acknowledgments. The nursing staff of the clinical research unit provided valuable assistance with care of the patients and collection of blood samples. Mr. Charles Burnham and Dr. Rene Gibson provided the bile acid measurements, Dr. Jerry Spenney provided the ^{125}I-cholylglycylhistamine used in the assay, and Dr. Stephen Barnes gave advice in the preparation of this manuscript.

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