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Postprandial serum bile acids in healthy man

Evidence for differences in absorptive pattern between individual bile acids

B. ANGELIN[1] AND I. BJÖRKHEM

From the Department of Medicine, Serafimerlasarettet, and Department of Clinical Chemistry, Huddinge Sjukhus, Karolinska Institutet, Stockholm, Sweden

SUMMARY The serum concentrations of cholic acid (C), chenodeoxycholic acid (CD), and deoxycholic acid (D) before and after a standardised meal were determined in five healthy female subjects using a highly specific and accurate gas chromatographic-mass spectrometric technique. The C level rose significantly 60 minutes after the meal, reached a peak after 90 minutes, and had returned to the original level after 150 minutes. In contrast, the serum concentrations of CD and D displayed a significant rise by 30 minutes, reached a peak after 90 minutes, but had not returned to fasting levels after 150 minutes. The serum bile acid responses after a meal suggest that there is considerable absorption of dihydroxy bile acids in the proximal small intestine in man.

In man, the primary bile acids, cholic acid (C) and chenodeoxycholic acid (CD), together with the secondary bile acid, deoxycholic acid (D), are secreted by the liver conjugated with glycine and taurine and efficiently absorbed from the intestine. The principal part of the absorptive process has been ascribed to the distal ileum, where an active transport of bile acids takes place. However, not only active transport but also ionic and non-ionic as well as micellar diffusion must be considered (Dietschy, 1968). We have recently shown that there is a considerable increase in the C/CD and C/D ratios in intestinal aspirates along the proximal intestine under physiological conditions in man. The most probable explanation is a substantial absorption of the dihydroxy bile acids at this level caused by non-ionic diffusion (Angelin *et al.*, 1976). The possibility of differences in absorptive patterns between individual bile acids is of importance for our understanding of the enterohepatic circulation and of the factors controlling the biliary secretion. We have therefore tried in the present work further to support this hypothesis by studying the response of individual serum bile acids to a meal. The serum bile acids have been determined by a highly specific and accurate gas chromatographic-mass spectrometric technique.

[1]Address for correspondence: Dr Bo Angelin, Department of Medicine, Serafimerlasarettet, S-112 83 Stockholm, Sweden.

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Methods

SUBJECTS AND EXPERIMENTAL PROCEDURE

Five healthy, non-obese female subjects with normal routine liver function tests and serum lipid levels volunteered for the study. Their mean age was 33 (range 24-53) years and their mean weight 62 (range 54-80) kg. They all had well-functioning gallbladders, as judged from oral cholecystography. The subjects presented in the morning at 7.30 after a 12 hour fast. An indwelling needle was inserted into an antecubital vein, and two to three blood samples were drawn in the fasting state. The needle remained in place during the study and was kept patent by intermittent flushing with small doses of isotonic saline. At 8.00 a.m. the subjects were given a standardised breakfast, consisting of 2 dl milk, two cheese sandwiches, and 2 dl coffee. The energy content of the meal is equivalent to 1750 kJ with carbohydrate, fat, and protein accounting for 33, 44, and 23% of the total, respectively. Five millilitres of venous blood were drawn from the indwelling catheter at 10 to 15 minute intervals for 150 minutes.

TECHNIQUES

A detailed description of the analytical procedure is to be published elsewhere (Angelin *et al.*, to be published) and only a brief description will be given.

After clotting of the venous blood at room temperature, serum was obtained by centrifugation and frozen at −20°C for later analysis.

To exactly 1 ml serum, 2·5 μg [2,2,3,4,4-$^2H_5$]C (dissolved in 25 μl acetone) and 5·0 μg [11,11,12-$^2H_3$]D (dissolved in 50 μl acetone) were added. The deuterated internal standards had been prepared by enolising methyl 3-oxo-7α, 12α-dihydroxy-5β-cholanoate and methyl 12-oxo-3α-hydroxy-5β-cholanoate respectively in an alkaline deuterated medium, followed by reduction with sodium borodeuteride and preparative thin-layer chromatography (*cf.* Hachey *et al.*, 1973). The serum, together with the internal standards, was then subjected to hydrolysis with 1 M KOH at 110°C for 12 hours. The alkaline solution was extracted three times with ethyl ether. The bile acids were then extracted from the acidified water phase with ethyl ether. The residue of the ether extract was methylated with diazomethane and converted into trimethylsilyl ether. The derivatives were analysed by gas chromatography-mass spectrometry using an LKB 9000 instrument equipped with an MID-unit (Multiple Ion Detector). A 1·5% SE-30 column was used. Three of the channels of the MID-unit were focused on the ions at m/e 368, 370, and 373. The ion at m/e 368 corresponds to the M − 3 × 90 peak in the mass spectrum of derivative of unlabelled C. The ion at m/e 370 corresponds to the M − 2 × 90 peak in the mass spectrum of derivative of unlabelled D and CD. The ion at m/e 373 corresponds to the M − 3 × 90 peak in the mass spectrum of [2,2,3,4,4-$^2H_5$]C and to the M − 2 × 90 peak in the mass spectrum of [11,11,12-$^2H_3$]D. [2,2,3,4,4-$^2H_5$]C was used as internal standard for unlabelled C and [11,11,12-$^2H_3$]D was used as internal standard for both unlabelled D and unlabelled CD. The concentrations of the individual bile acids were thus calculated from the ratios C/[2,2,3,4,4-$^2H_5$]C (tracing at m/e 368/tracing at m/e 373), D/[11,11,12-$^2H_3$]D (tracing at m/e 370/tracing at m/e 373) and CD/[11,11,12-$^2H_3$]D (tracing at m/e 370/tracing at m/e 373). Standard curves were used for each individual bile acid. The relative standard deviation of the method as calculated from

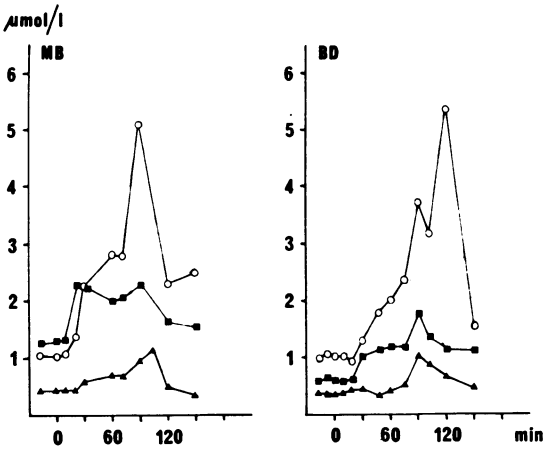

Fig. 1 *Concentrations of individual serum bile acids (μmol/l) in two representative subjects, MB and BD. At time 0 a standardised breakfast was given and the serum levels were followed for 150 minutes.* ▲—▲ *cholic acid (C),* ■—■ *deoxycholic acid (D),* ○—○ *chenodeoxycholic acid (CD).*

$$\left(100\sqrt{\frac{\sum\left(2\frac{a-b}{a+b}\right)^2}{n-1}}\right),$$

duplicate samples, was about 6% for each bile acid (Angelin *et al.*, to be published). Recovery experiments have shown that the same results are obtained in the assay regardless of whether free or conjugated bile acids are added.

Results

The fasting values for C, CD, and D were 0·46 ± 0·06, 1·06 ± 0·11, and 1·22 ± 0·21 μmol/l, respectively (means ± SEM, Table). In all five subjects, C showed an increase from the basal level, beginning after about 60 minutes and reaching a clear peak after 90-120 minutes. After 150 minutes the C concentration had returned to the

Table *Serum concentrations (μmol/l) of cholic (C), chenodeoxycholic (CD), and deoxycholic (D) acids before and at 30, 60, 90, 120, and 150 minutes after standardised meal*

	0	*30*	*60*	*90*	*120*	*150*
C	0·46 ± 0·06	0·56 ± 0·11	0·74 ± 0·07[b]	1·01 ± 0·07[c,d,e]	0·72 ± 0·17	0·57 ± 0·13[f]
CD	1·06 ± 0·11	2·15 ± 0·31[b]	2·44 ± 0·40[a]	3·04 ± 0·23[c]	2·41 ± 0·32[a]	1·80 ± 0·21[a,f]
D	1·22 ± 0·21	1·92 ± 0·32[b]	1·91 ± 0·34[a]	2·24 ± 0·26[a]	2·01 ± 0·23[b]	1·65 ± 0·18[f]

Significantly different (Student's paired *t* test) from [a]t = 0, P < 0·05, [b]t = 0, P < 0·01, [c]t = 0, P < 0·001, [d]t = 30, P < 0·05, [e]t = 60, P < 0·05, [f]t = 90, P < 0·05.

The figures express means ± SEM of five healthy female subjects.

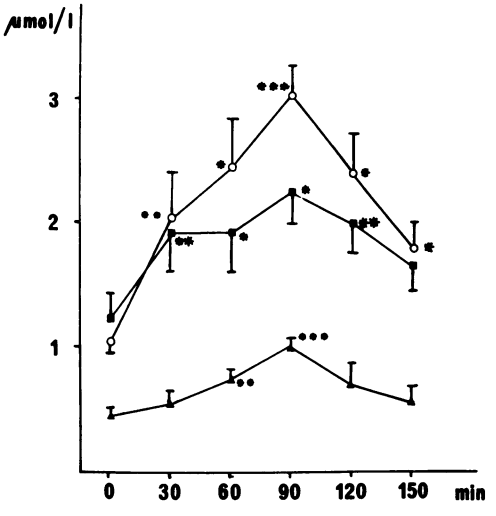

Fig. 2 *Response to a standardised meal of individual bile acids in serum. The means of five healthy subjects are given for each bile acid, and the bars indicate SEM. Observations significantly different from the fasting state (by Student's paired* t *test) are shown as* $*P < 0.05$, $**P < 0.01$, *and* $***P < 0.001$. ▲—▲ *cholic acid (C),* ■—■ *deoxycholic acid (D),* ○—○ *chenodeoxycholic acid (CD).*

initial values in all subjects. The consecutive values for two representative subjects, MB and BD, are shown in Fig. 1. The values 60 and 90 minutes after the meal differed significantly from the basal level (Fig. 2, Table).

In contrast with this pattern, the serum levels of CD showed a rapid increase over the first 60 minutes, followed by a still more impressive peak coinciding with that of C. The values 30, 60, 90, 120, and 150 minutes all differed significantly from those in the fasting state (Table, Fig. 2), and thus, unlike the behaviour of C, the CD concentration did not return to basal levels during the study. A similar pattern was seen for the serum concentration of D (Figs. 1 and 2, Table).

Discussion

Using these methods, it has been possible to measure all three major bile acids in human serum—that is, C, CD, and D (Angelin *et al.*, to be published). Similar techniques based on specific ion monitoring (mass fragmentography) with use of isotope labelled internal standards have been published previously (Klein *et al.*, 1974; Miyazaki *et al.*, 1974), but so far as we know they have so far been used only to a very limited extent for determining the level of bile acids in serum or bile under different conditions. The technique should be superior to previous methods based on gas-chromatography (Sandberg *et al.*, 1965) as far as accuracy is concerned. Thus, in order to interfere with the mass fragmentographic assay, a compound must have the same retention time in the gas chromatography and contain the same characteristic fragment in the mass spectrum as the compound to be determined. A great advantage in the assay is the fact that the deuterium labelled internal standard is added to the serum before hydrolysis and extraction. As the difference between the bile acid and the corresponding internal standard is only a few mass units, any loss in the extraction or derivatisation must affect the internal standard to the same extent as the unlabelled bile acid. Preferably, the internal standards should have been conjugated with taurine or glycine. There is no reason to believe, however, that use of free bile acids as standards may affect the assay. It was shown in recovery experiments that the same results are obtained regardless of whether free or conjugated bile acids are added to a serum. Recently, a radioimmunoassay technique for conjugated cholyl bile acids in serum has been developed (Simmonds *et al.*, 1973) and a preliminary report on a CD assay has appeared (Schalm *et al.*, 1975). However, in the latter study a discrepancy was found between the values obtained with the radioimmunological technique and a gas chromatographic technique, probably due to cross-reactivity.

In the present work, the bile acids were analysed as free bile acids after deconjugation. This procedure makes it impossible to separate different conjugates or unconjugated bile acids from each other. Furthermore, sulphated bile acids, accounting for about 9% of total serum bile acids (Makino *et al.*, 1975) are not measured with the present method. However, these limitations should be of minor importance in the present study.

The serum concentration of a specific bile acid is mainly determined by the momentary balance between the 'input' from intestinal absorption and the 'output' caused by hepatic clearance. Other ways of elimination such as renal excretion seem to be of minor importance in healthy man (Cowen *et al.*, 1975). After a meal, there was a clear-cut rise in serum C concentration which reached a peak after 90-120 minutes. This is in accordance with the results of LaRusso *et al.* (1974), and is interpreted as a 'spillover' from the hepatic extraction during the maximal portal inflow caused by ileal absorption. The behaviour of the dihydroxy bile acids, CD and D, was clearly different from that of C. The maximum peak in serum concentration parallelled that of C, but there was a definite gradual increase over the first 60 minutes, during which the C levels were unchanged. A possible explanation for the difference is

a less efficient hepatic clearance of CD (and D). Thus, the half-life of conjugated CD in serum has been reported to be somewhat longer than that of C conjugates (Cowen *et al.*, 1975). The extreme rapidity of the clearance of both bile acids, however, makes it difficult to ascribe the present findings entirely to differences in hepatic clearance. Another explanation is then that after a meal there is an increased 'input' of CD and D from the intestine, presumably because of passive absorption of the glycine conjugates (Switz *et al.*, 1970). This agrees with our previous study, in which jejunal absorption of the dihydroxy bile acids was calculated to be about 30% (Angelin *et al.*, 1976). The slow return of CD and D serum levels to the basal values could be ascribed to a continued enhanced small intestinal 'input', possibly caused by recirculation of the bile acid pool.

During the course of the present study Schalm *et al.* (1975), in abstract form, reported results similar to those obtained by us. These authors used a similar experimental model but measured both C and CD by the radioimmunological technique mentioned above.

The presence of a dihydroxy bile acid 'short-cut' of the hepatoileal circuit explains the observed differences between the C/CD ratio in duodenal bile and the C/CD pool size ratio (Angelin *et al.*, 1976). From experiments in the rat, it has been suggested that different bile acids may have different effects upon cholesterol and fat absorption (Holt, 1972) as well as feed-back regulation of cholesterol biosynthesis and degradation (Danielsson, 1973; Shefer *et al.*, 1973). In man, feeding of C and CD have different effects on the secretion of cholesterol (Adler *et al.*, 1975; LaRusso *et al.*, 1975). The differences in absorptive patterns between individual bile acids suggested by the present work may be of some importance for our understanding of the regulation of the enterohepatic circulation of bile acids and of the factors influencing the biliary secretion of lipids.

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