Physiological factors influencing serum bile acid levels

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SUMMARY This study defines the effects of fasting (prolongation of an overnight fast for a further four hours), feeding (the response to eating the three main 'solid' meals of the day), and cholecystokinin-induced gallbladder contraction (75-100 units of CCK given as a bolus intravenous injection) on serum individual bile acids in five to eight healthy control subjects. The serum conjugates of the two primary bile acids, cholic and chenodeoxycholic, were measured using sensitive specific radio-immunoassays. During fasting, there was no significant change in the levels of the serum individual bile acids (conjugates of cholate, 1·28 ± 0·19; conjugates of chenodeoxycholate, 1·17 ± 0·17 μmol/l). After breakfast, the serum conjugates of cholate and chenodeoxycholate increased significantly but thereafter the mean values remained high with less consistent responses to lunch and dinner, some subjects showing a peak and trough response to all three meals, while others showed a plateau response throughout the day. After breakfast, the serum chenodeoxycholate conjugates increased more rapidly (peak at 60 minutes when the concentration reached 2·07 ± 0·30 μmol/l) and to a greater extent than the conjugates of cholate (peak at 90 minutes; 1·50 ± 0·24 μmol/l). A similar pattern of results was seen after intravenous CCK, suggesting either preferential jejunal absorption of chenodeoxycholate conjugates and/or preferential hepatic clearance of cholate conjugates. These results provide essential background data for future studies of serum individual bile acids in intestinal and hepatic disease.

Bile acids in the body are almost entirely confined to the enterohepatic circulation by two efficient transport systems, one in the intestine (Lack and Weiner, 1961; Krag and Phillips, 1974) and the other in the liver (Reichen and Paumgartner, 1975, 1976; Glasinović et al., 1975). Some 95-99% of the bile acids secreted into the intestine in bile are reabsorbed (Dowling et al., 1970) returning to the liver in the portal vein. The hepatic uptake system then removes most of the bile acids from the sinusoidal blood in a single 'circulation pass' (O'Maille et al., 1967; Hoffman et al., 1975; Gilmore et al., 1977). Normally, only small quantities of bile acids escape past the hepatic uptake mechanism to spill into the peripheral circulation (LaRusso et al., 1974), and therefore systemic bile acid concentrations are usually maintained at very low levels (Carey, 1958; Sandberg et al., 1965; Roovers et al., 1968; Panveliwalla et al., 1970; Bell et al., 1974).

Several studies have shown that in many patients with liver disease, both fasting (Sherlock and Walsh, 1948; Rudman and Kendall, 1957; Neale et al., 1971; Bloomer et al., 1976) and postprandial (Kaplowitz et al., 1973; Barnes et al., 1975; Fausa, 1976; Fausa and Gjone, 1976) serum bile acid levels rise. Indeed, it has been suggested that raised serum bile acids are among the most sensitive markers of liver disease (Frosch and Wagener, 1967; Barnes et al., 1975; Isaacs et al., 1976). This conclusion is based mainly on measurements of serum total bile acids but in a few studies the diagnostic value of serum individual bile acids has also been stressed (Korman et al., 1974; Javitt and Levy, 1975; Demers and Hepner, 1976; Isaacs et al., 1976). However, before one can interpret increased serum bile acid levels in liver disease, one must know something about individual serum bile acid concentrations in health and how these may be influenced by physiological events. But surprisingly little is known about the factors influencing normal serum bile acid levels—mainly for technical reasons, as the sensitivities of spectrophotometric (Carey, 1958), sophisticated thin layer chromatographic (Panveliwalla et al., 1970; Neale et al., 1971), enzymatic fluorimetric (Murphy et al., 1970; Schwarz et al., 1974), and gas-liquid chromatographic methods.
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(Sandberg et al., 1965; Ali and Javitt, 1970) are stretched to their limits to measure individual serum bile acids in health.

The advent of sensitive and specific radioimmunoassays, which can accurately measure concentrations as low as 0·1 μmol/litre, has now made measurement of individual serum bile acid levels in normal sera possible. In the present studies, two such assays, developed in our laboratories, to measure the conjugates of both cholic (c-CA; Murphy et al., 1974) and chenodeoxycholic9 (c-CDCA; Murphy et al., 1976) acids have been used to study the influence of a brief fast, of feeding, and of gallbladder contraction on serum bile acid levels in health.

Methods

EXPERIMENTAL DESIGN

After an overnight fast, a baseline blood sample was taken at 09.00 hours and then further samples were withdrawn at 30 or 60 minute intervals over the next four to 12 hours.

In the fasting studies, blood samples were withdrawn at 60 minute intervals when the overnight fast was prolonged for a further four hours. These samples were obtained to provide the most appropriate control values for comparison with the serum bile acid responses to breakfast and to CCK.

For the 12 hour serum bile acid profile, blood samples were withdrawn at hourly intervals throughout the day (with the exception when samples were taken at 30 minute intervals for the first two hours) during which the subjects ate the three main meals, the composition of breakfast, lunch, and dinner being standardised and comparable from subject to subject.

As food may influence serum bile acid levels through the release of endogenous cholecystokinin (CCK) with subsequent gallbladder contraction, we compared the results obtained after food with those found after a bolus injection of 75-100 units of cholecystokinin (Boots' Pancreozymin or GIH Laboratories' CCK, Dr Jorpes, Karolinska Institute, Stockholm, Sweden) given intravenously over a 10 minute period.

SUBJECTS AND PATIENTS STUDIED

Effect of fasting

This was studied in six men, aged 24-39 years, all of whom were healthy control subjects.

Effect of food

Of the eight subjects in this study, five (who had also been studied during the four hour fast) were male members of staff aged 24-39 years. The remaining three (two men aged 44 and 59 years and one woman aged 62 years) were patient volunteers who were convalescing after attacks of chest pain. None had gastrointestinal or liver disease and all had normal conventional tests of liver function.

The standardised solid meals were taken at 09.00, 13.00, and 18.00 hours. Breakfast consisted of an egg, two slices of bread and butter, and a cup of white coffee. Lunch and dinner consisted of meat, vegetable and potatoes, and a dessert.

Effect of CCK-induced gallbladder contraction

Serum bile acid levels were measured at 30 minute intervals after the end of a 10-minute intravenous injection of CCK given to eight subjects, six of whom were laboratory personnel (five men aged 24-33 years and one 23 year old woman—three of whom had also part taken in the four hour fasting study). There were two male patient volunteers (aged 46 and 56 years) both convalescing from cardiovascular disease, who were well at the time of study and who again had no evidence of liver or intestinal disease.

TECHNIQUES

Serum bile acid analyses

The blood samples were allowed to clot at room temperature; the serum was then separated and stored at 4°C until analysed.

With the radioimmunoassay methods used to measure the conjugates of both cholic and chenodeoxycholic acid (Murphy et al., 1974, 1976) 0·1 ml of a 1:20 dilution of serum was incubated in duplicate with isotopic antigen and antibody and after precipitation with (NH₄)₂SO₄, the radioactivity in 0·3 ml of the reaction mixture was counted (again in duplicate) in an LKB Wallac 81000 liquid scintillation counter using 10 ml of a commercial scintillation fluid (New England Nuclear, NE 260) with external standard quench corrections. The error between duplicates was less than 10%.

Previous studies from our laboratory have shown that when measuring the conjugates of cholic and chenodeoxycholic acids, the 'cross-reactivity' with other conjugated bile acids at 50% binding is small (0-10%) and, although unconjugated cholic and chenodeoxycholic acids may cross-react with their respective conjugates, this cross-reactivity never exceeds 10% (Murphy et al., 1974, 1976).

Statistical methods

The results are expressed as mean values with standard errors of the mean. In all the studies, the fasting baseline values were used as a reference point.

In this paper the trivial names for 3α-, 7α-, 12α-trihydroxy-5β-cholanic acid (cholic acid) and 3α-, 7α-dihydroxy-5β-cholanic acid (chenodeoxycholic acid) have been used.
for comparison with post-prandial and post-CCK serum bile acid levels and the significance of differences between the means estimated using Student's paired t test. A comparable pattern of statistical significance was found when the results were analysed with the Wilcoxon Rank Sum test for non-parametric data.

Results

SERUM BILE ACID LEVELS

Effect of fasting

These data are shown in the middle panel of Fig. 1. After an overnight fast, the mean value for the conjugates of cholic acid was 1.28 ± 0.19 μmol/l and for chenodeoxycholic acid, 1.17 ± 0.17 μmol/l. Over the next four hours, the mean levels remained essentially unchanged, although, if anything, the concentrations of both individual bile acid groups tended to fall with time.

Effect of food

As Fig. 2 shows, serum cholic and chenodeoxycholic acid conjugates increased after breakfast and thereafter, although the results in individual subjects fluctuated considerably, the mean values tended to remain high throughout the day. For this reason the serum bile acid responses to food after an overnight fast (breakfast—Fig. 1, upper panel, when more frequent samples were taken) and those seen throughout the day (which also includes the response to lunch and dinner—Fig. 2) are considered separately.

After breakfast (Fig. 1), the mean serum conjugates of both cholic and chenodeoxycholic acids showed significant increases when compared with fasting baseline values. However, the response of the two bile acid groups was different: the serum chenodeoxycholate conjugates rose more quickly and to higher levels so that the 30 minute value of 1.70 ± 0.30 μmol/l was already significantly greater than the levels seen after an overnight fast (0.70 ± 0.10 μmol/l; t = 2.240, P < 0.02). By one hour, both serum bile acid groups were significantly greater than their corresponding levels at time zero but the 230% increase in the conjugates of CDCA was relatively much greater than the 85% increase in cholate conjugates, which did not reach a peak until 90 minutes after breakfast.

In contrast, the response to lunch and dinner was less clear cut (Fig. 2). The mean serum conjugates of cholate rose from 1.16 ± 0.25 μmol/l before lunch (at four hours after starting the 12 hour profile) to 1.88 ± 0.28 μmol/l one hour later, and, although this difference was not statistically significant, the 38% increase was nearly three times greater than the 13% increase in the corresponding serum chenodeoxycholate conjugates. There was a similar discrepancy in the hour after dinner but this time the conjugates of chenodeoxycholic acid showed a more marked (53%) and statistically significant (t = 2.234; P < 0.025) rise from hour 9 to hour 10 than the mean serum conjugates of cholic acid measured at the same time.

The scatter of results for the conjugated bile acids during the 12 hour profile is emphasised by the comparatively large SEMs seen in Fig. 2. This is partly due to marked individual variations in the serum bile acid responses to food and this is clearly illustrated by representative examples of different patterns of response in two different subjects in Fig. 3. In subject 1 (the solid line in Fig. 3), there was a clear peak and trough response to all three meals—a pattern similar to that seen in three other subjects studied—while in subject 2 (the broken line in Fig. 3), although the serum individual bile acid levels increased gradually after breakfast, thereafter there was little change throughout the day—the type of response seen in the three remaining subjects in this group.

Effect of CCK-induced gallbladder contraction

The serum cholate and chenodeoxycholate conjugate
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Fig. 2  The serum conjugates of chenodeoxycholic acid (upper panel) and cholic acid (lower panel) measured over 12 hours, during which the eight subjects ate the three main meals of the day (mean values ± SEMs). *Mean value significantly different from baseline value at time zero (p < 0.05). **Mean value significantly different from baseline value at time zero (p < 0.01).

Fig. 3  Representative examples of the 12 hour 'profiles' for the serum conjugates of cholic and chenodeoxycholic acids seen in two different subjects (see legend to Fig. 2).

responses to intravenous CCK are shown in Fig. 1 (lower panel).

As with the response to breakfast, the serum chenodeoxycholic acid conjugates again showed a greater response to cholecystokinin than the serum conjugates of cholic acid. However, with the rapid stimulus of an intravenous injection (as opposed to the more prolonged stimulus during gastric emptying) the increase in serum chenodeoxycholic acid conjugates was earlier and more short-lived than the response to breakfast (Fig. 1, upper panel). The peak value in serum chenodeoxycholic acid conjugates of 2.15 ± 0.45 μmol/l, 30 minutes after CCK, was comparable to the peak level of 2.07 ± 0.30 μmol/l seen 60 minutes after breakfast. The serum cholate conjugates also showed a brisker response to cholecystokinin than to breakfast, but the post-CCK profile was again flatter and more protracted than that seen for serum CDCA conjugates.

Discussion

These studies have defined the serum response of the conjugates of the two primary bile acids, cholate and chenodeoxycholate (normally the major bile acids present in serum), to fasting, feeding, and gallbladder contraction using recently developed, sensitive and specific radioimmunoassay techniques.

Previous studies of serum individual bile acids

There have been comparatively few studies of serum
individual bile acids in health, almost certainly because, until recently, existing methods were too insensitive. Panveliwalla et al. (1970) used elegant but complex and time-consuming thin-layer chromatographic techniques to separate the individual bile acids in serum. Since their serum total bile acid concentration was only 2.3-3.6 μmol/l, only the most fastidious could achieve reproducible results for serum individual bile acid levels with this method. GLC methods will reliably measure serum individual bile acid levels in various types of liver disease (Roovers et al., 1968; Kaplowitz et al., 1973), but with recoveries as low as 60% (Sandberg et al., 1965) this method is probably too insensitive to measure accurately serum individual bile acids in health.

Radioimmunoassay techniques, comparable to those used in the present study, have been applied by others to estimate serum individual bile acids in health. In a recent report of individual serum bile acid levels in various types of liver disease, Demers and Hepner (1976) included normal values for the glycine conjugates of cholic, deoxycholic, and chenodeoxycholic acids in 21 control subjects. LaRusso and colleagues (1974) and Schalm et al. (1975) showed in four to five subjects that the serum conjugates of both chenodeoxycholic and cholic acids showed a well demarcated peak and trough response to food—similar to that illustrated by subject I in Fig. 3. However, these authors studied the response to liquid formula meals and we would suggest that the more variable pattern of results seen over a 12 hour period in the present investigation is more representative of the normal day-to-day pattern in subjects eating 'solid' meals.

**Significance of Present Results**

**Serum bile acids during fasting**

As hepatic clearance of both isotopic (Blum and Spritz, 1966; Klapdor, 1971; Kaye et al., 1973; Cowen et al., 1975; Isacca et al., 1976) and non-isotopic bile acids (O'Maille et al., 1967; Korman et al., 1975) is extremely rapid, one might reasonably ask why any measurable bile acids are present in the peripheral blood during fasting. There seem to be at least two possible explanations. First, the traditional concept that after an overnight fast the bile acid pool is almost completely stored in the gallbladder may not be true. In fact, a recent study in the hamster (Ho, 1976) has shown that, after a 12 hour fast, only 17% of the bile acids are present in the gallbladder, the majority of the pool being in the small intestine. Furthermore, preliminary studies in man (von Bergmann et al., 1976) have suggested that at the end of an overnight fast, the gallbladder contains, on average, only 58% of the total bile acid pool. By implication, therefore, considerable quantities of bile acids must be present in the human small intestine during fasting. If so, they are likely to be absorbed, spilling past the hepatic uptake mechanism to appear in the peripheral blood. Secondly, it is known that bile acids may be absorbed, albeit poorly, from the colon (Samuel et al., 1968) and that normally a minimum of 250-500 mg of bile acids per day must have escaped past the ileal uptake mechanism to pass through the colon since this amount appears in the faeces (Grundy et al., 1965; Evrard and Jansen, 1968). During fasting, therefore, there may well be a continuous trickle of bile acids arriving in the peripheral blood which have been absorbed from the colon and which in turn have by-passed the hepatic bile acid transport mechanism. However, serum bile acids of colonic origin are likely to be unconjugated bile acids. To date, there has been only one study which suggested that unconjugated bile acids may be found in fasting serum (Makino et al., 1969) and there have been no studies of serum unconjugated bile acid levels after meals. For this reason, the colonic absorption hypothesis is unlikely to explain the results for the serum bile acid conjugates.

**Response to food**

Two aspects of the present results of serum individual bile acid responses to food merit comment—the different response to breakfast from that seen after subsequent meals and the fact that the serum conjugates of chenodeoxycholic acid showed a more rapid and greater rise after food than the serum conjugates of cholic acid.

The reason why the serum bile acids in some subjects studied did not return to the baseline values (those seen after an overnight fast) between meals may be due to the fact that gastric emptying of 'solid' food is slower than that of liquid test meals (Heading et al., 1976). Solid food may cause a more prolonged release of endogenous CCK, a more protracted delivery to the intestine of bile acids from the gallbladder, and more prolonged absorption of the bile acids from the gut. If this hypothesis is correct, it could explain why there was a clear-cut serum individual bile acid response to breakfast taken after 12 to 15 hours of fasting and a much less consistent response to lunch and dinner.

The observation that the rise in serum chenodeoxycholic acid conjugates after meals is earlier and greater than that for serum cholate conjugates has been made before (Schalm et al., 1975; Angelin et al., 1976), but the explanation for this phenomenon is by no means clear. As gallbladder contraction must expel both types of bile acid conjugates...
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simultaneously, we are left with two possible explanations. First, that the conjugates of the dihydroxy bile acid, chenodeoxycholic acid, are absorbed from the jejunum more readily than the conjugates of the trihydroxy bile acid, cholic acid, or, secondly, that there is differential hepatic clearance.

With regard to jejunal absorption, the results of several studies both in animals and in man have shown indirectly that chenodeoxycholate is better absorbed from the upper small bowel than cholate. Schiff et al. (1972) showed that, in the rat, the passive non-ionic jejunal diffusion of chenodeoxycholate was greater than that of cholate, while, in man, Angelin et al. (1976) found that the ratio of cholate to chenodeoxycholate remaining in the intestinal lumen increased progressively from proximal to distal small bowel, suggesting that there was preferential and selective reabsorption of the dihydroxy bile acid. Furthermore, Hislop et al. (1967), using segmental perfusion studies in man, showed that conjugated dihydroxy bile acids were absorbed more rapidly than the conjugates of cholate. The earlier rise in chenodeoxycholic acid may, therefore, be explained, in part at least, by preferential jejunal absorption.

With regard to hepatic clearance, studies in man (Cowen et al., 1975) have shown that, while the conjugates of cholic and chenodeoxycholic acid are both extracted rapidly by the liver, the clearance of the chenodeoxycholic acid conjugates is less efficient. It is possible, therefore, that differential hepatic uptake contributes to the greater rise in chenodeoxycholic acid conjugates seen after both food and CCK.

Response to gallbladder contraction

The serum individual bile acid response to CCK-induced gallbladder contraction shows both similarities to and differences from the response to food. The greater and more rapid rise in serum chenodeoxycholate conjugates after intravenous CCK has not previously been described. In this respect, the response to gallbladder contraction is similar to that seen after food. It seems likely that the same mechanism proposed to explain the differential post-prandial rise in serum cholate and chenodeoxycholate levels also applies to gallbladder contraction and supports the concept that the increase in serum individual bile acids seen after eating is indeed due to gallbladder contraction.

The differences in the response to food and to intravenous CCK lie in the speed of change of serum bile acids—particularly for serum chenodeoxycholic acid conjugates, which reached a peak only 30 minutes after CCK and returned to pre-injection baseline levels 90 minutes later. This more rapid rise-and-fall pattern is probably due to the short-lived stimulus to gallbladder contraction seen after a bolus injection of CCK as opposed to (as discussed above) the more protracted stimulus as a result of solid food leaving the stomach over a two hour period. Isotope scanning studies of gallbladder contraction with doses of CCK similar to those used in the present study have shown that the mean lag time before gallbladder contraction begins is 3.5-5.0 minutes after giving the intravenous CCK and the mean t½ for gallbladder contraction is 6.5-7.5 min (Bingham and Maisey, 1977). Although CCK also stimulates small bowel motility (Hedner et al., 1967) and promotes more rapid transit of intestinal contents (Dollinger et al., 1975), the fact that the peak rise in serum chenodeoxycholic acid conjugates was seen within 30 minutes again suggests that these bile acids must be being absorbed in the jejunum (as well as in the ileum).

In conclusion, we would suggest that the results of these studies provide essential background information which should enable us to understand more fully the underlying mechanisms for the increased serum bile acid levels found in different types of intestinal and liver disease.

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