Assessment of gall-bladder storage function in man

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(Received 17 August 1982/16 February 1983; accepted 24 February 1983)

Summary

1. We have validated a scintiscanning method for measuring fasting-state gall-bladder (GB) filling in man. 99mTc-labelled diethyl phenylcarbamoylmethyliminodiacetate (Tc-HIDA) was given intravenously, and 90 min later GB and gut activity were measured by using two isosensitive rectilinear scanning heads (anterior and posterior). Studies with a phantom GB in vitro, and studies in man in vivo, showed that the maximum error due to differences in isotope depth was 8%, compared with 300% when only one head was used.

2. By combining this technique with measurement of biliary lipid concentrations of fasting-state GB bile obtained by nasoduodenal intubation and intravenous cholecystokinin infusion, we were able to measure for the first time the total mass of all three biliary lipids in the GB. GB bile samples obtained in this way were divided into three consecutive portions of equal size in order to assess GB mixing. Bile acid pool size was also measured by isotope dilution.

3. We studied 12 healthy non-obese men. Fasting-state GB filling over 90 min (mean ± SEM) was 54 ± 8%. Biliary lipid mass in GB was 4.9 ± 0.5 mmol for bile acids (67 ± 5% of the total bile acid pool), 1.6 ± 0.2 mmol for phospholipid and 0.5 ± 0.1 mmol for cholesterol. The three consecutive portions of fasting GB bile gave values of 1.05 ± 0.07, 1.05 ± 0.06 and 1.03 ± 0.10 for cholesterol saturation index (SI) and 6.6 ± 1.1, 7.4 ± 1.6 and 6.5 ± 1.0 for Tc-HIDA c.p.m. x 1000 per mmol of bile acids.

4. The SI of fasting-state GB bile was significantly correlated with fasting-state GB filling (r = 0.63; P < 0.05). It was also correlated with cholesterol mass in GB (r = 0.64; P < 0.05), but not with bile acid and phospholipid mass.

5. We conclude that: (a) valid measurements of GB filling can be made in man by a simple scintiscanning technique employing 99mTc-HIDA as a biliary marker; (b) biliary lipid mass can also be measured if GB bile is obtained; (c) SI in health is in part determined by the degree of fasting-state GB filling, and in part by cholesterol mass in GB; (d) fasting-state GB content is well mixed in health.

Key words: bile acids, cholesterol saturation index, gall-bladder.

Abbreviations: GB, gall-bladder; SI, cholesterol saturation index; BA, bile acids; Tc-HIDA, 99mTc-labelled diethyl phenylcarbamoylmethyliminodiacetate.

Introduction

Patients with cholesterol gallstones have fasting-state GB bile that is supersaturated with cholesterol more frequently than in controls without gallstones [1-7]. In order to elucidate the factors influencing SI of fasting-state GB bile, we have developed and validated a technique for the simultaneous assessment of a variety of GB storage functions, and have applied this technique to determine the contribution of these functions to the SI of fasting-state GB bile in health.

Hepatic bile [8] is supersaturated with cholesterol during overnight fasting even in health, as a result of physiological interruption of the enterohepatic circulation of BA. Thus the degree of GB filling with fasting-state hepatic bile (defined as the proportion of hepatic bile sequestered into the GB over a defined period) could influence the SI of GB bile. We have therefore developed and validated a radioisotopic method of measuring
fasting-state GB filling, and have correlated this measurement with the SI of fasting-state GB bile in a group of 12 healthy volunteers.

The conventional technique of collecting fasting-state GB bile by nasoduodenal intubation allows calculation only of relative biliary lipid composition [1, 9, 10]. This does not indicate in absolute terms whether supersaturated GB bile is due to an increase in cholesterol mass, or to a decrease in BA and/or phospholipid mass stored in the GB. Although indirect information is available from biliary lipid secretion rates, obtained by using duodenal perfusion techniques [11-13], these have given conflicting results and may be subject to artifacts. We have therefore developed a simple and reliable method for measuring the mass of all three biliary lipids in fasting-state GB bile by combining nasoduodenal intubation with our isotope scanning technique.

It has been suggested that stratification of GB contents might allow sequestration of fasting-state hepatic bile, supersaturated with cholesterol, in which localized crystal precipitation might initiate gallstone formation [14]. We have assessed mixing of GB contents by analysing three sequential portions of GB bile for consistency of SI as an index of long-term mixing, and of the $^{99m}$Tc-HIDA/BA ratio measured 3 h after an intravenous injection of $^{99m}$Tc-HIDA as an index of short-term mixing.

We have measured fasting-state GB volume by using ultrasound, a method that has been validated by Everson and colleagues [15]. From this and BA mass we have derived the BA concentration in GB. This is not otherwise possible for GB bile obtained by nasoduodenal intubation because of dilution by pancreatic and other secretions.

**Methods**

**Subjects**

Twelve healthy non-obese male volunteers were studied, age (mean ± SEM) 39 ± 4 years, weight 72 ± 4 kg, height 171 ± 2 cm and % ideal body weight 99 ± 3% (range 86-120%). They were all seen in a gastroenterology outpatient clinic complaining of abdominal pain, but no evidence of organic disease was found on gastrointestinal investigations including upper gastrointestinal endoscopy and either ultrasound examination or an oral cholecystogram for exclusion of gallstones. They all gave written informed consent. The studies were approved by the local Hospital Ethical Committee.

**Clinical procedures**

At 09.00 hours after a 12 h fast, the subjects received an intravenous injection of 1 mCi of $^{99m}$Tc-HIDA (Amersham International PLC, Amersham, Bucks, U.K.), a radio-pharmaceutical that is rapidly taken up by the liver and excreted in the bile. At the same time, the subjects had an intravenous injection of 5 μCi of [14C]chenodeoxycholic acid (Amersham International PLC). The volume of the GB and its distance from the anterior abdominal wall were determined in nine out of the 12 subjects by ultrasound (Unirad EDP 1000 static scanner) by using the method of multiple cylinders [15]. At 10.30 hours (90 min after the initial injection of Tc-HIDA) an abdominal cholescintigram was obtained with the subject in the supine position, with the use of a double-headed rectilinear scanner with area-of-interest facility (Fig. 1). The Tc-HIDA radioactivities (c.p.m.) over total abdomen, GB area and gut area (defined as total abdomen less GB, liver and urinary bladder areas) were recorded. A double-lumen poly(vinyl chloride) nasoduodenal tube was then passed and directed under X-ray screening to the duodenjejunal flexure. GB samples were collected after GB contraction was stimulated by a slow intravenous infusion over a period of 20-30 min of 100 Ivy Dog units of cholecystokinin (Pancreozymin; Boots Ltd, Nottingham, U.K.). Three or four consecutive samples of equal volumes of bilerich duodenal aspirate were collected and counted separately for Tc-HIDA radioactivity by using a γ-radiation counter, and the radioactivity of one of the bile samples was counted also by using the double-head rectilinear scanner. The GB bile sample was placed at approximately the same distance from the anterior scanner head as that of the
subject's GB in vivo, as assessed by ultrasound examination. This sample was used later to determine biliary lipid mass in the GB (see under 'Calculations'). All samples were retained for further laboratory analysis.

**Laboratory methods**

Biliary lipid concentration was determined by using enzymic analysis of the bile samples for BA [16], phospholipids [17] and cholesterol [18]. SI was determined in accordance with the criteria of Hegardt & Dam [9] and Holzbach et al. [2], with use of the polynomial equation of Thomas & Hofmann [10].

For determination of BA pool size, the bile samples were extracted with ethanolic 1% ammonia solution and subjected to thin-layer chromatography to separate the glycine and taurine conjugates of chenodeoxycholic acid [with chloroform/ethanol/acetic acid/water (12:8:4:1, by vol.) as solvent system]. After pooling of the glycine and taurine conjugates, 14C radioactivity was determined by liquid-scintillation counting and mass by enzymic assay in order to obtain the specific radioactivity. This 'one-shot' method for measuring BA pool size has been shown by Pomare & Low-Beer [19] to correlate well with the conventional Lindstedt method.

GB mixing was determined by collecting successive portions of GB bile into different containers. Each sample was counted for Tc-HIDA radioactivity and later analysed for BA concentration and SI (see other measurements). The ratio of Tc-HIDA radioactivity to BA concentration was taken as an index of short-term mixing of overnight GB contents with Tc-HIDA given only 3 h beforehand. At the same time we were able to assess long-term GB mixing by comparing SI values in the different samples.

**Calculations**

GB filling (%) was defined as follows:

$$\text{GB filling (\%)} = \frac{\text{GBc}}{\text{GBc} + \text{gut}_c} \times 100$$

where GBc is the Tc-HIDA radioactivity in the GB, and gutc is the Tc-HIDA radioactivity in the gut.

Biliary lipid mass in GB was determined by using the following relationship:

$$\frac{\text{GBm}}{\text{GBc}} = \frac{\text{Sm}}{\text{Sc}}$$

where Gbm is the mass of BA, phospholipids or cholesterol in GB, Sm is the mass of BA, phospholipids or cholesterol in the sample, and Sc is the Tc-HIDA radioactivity in the sample.

BA concentration in GB was obtained by dividing BA mass in GB by GB volume.

**Statistical analysis**

All results are expressed as means ± SEM. Coefficient of linear correlation (r) was calculated where appropriate.

**Results**

**Validation studies in vitro**

In order to validate the measurements obtained with the double-headed rectilinear scanner, a phantom GB was designed. This comprised a plastic water tank 25 cm x 25 cm x 25 cm. A small glass vial was suspended inside, this containing a dose of Tc-HIDA. In all studies the distance between the two heads of the scanner was kept constant. The following studies were performed with the phantom GB.

(a) The radioactivities of gradually decreasing doses of Tc-HIDA were counted with the use of both scanner heads. This was done by counting the radioactivity of the Tc-HIDA container after withdrawing each one of six identical small doses. There was a very good correlation between the dose of Tc-HIDA and the radioactivity counts (r = 0.998; P < 0.001).

(b) The distance of the glass vial from the anterior scanner head was changed by bringing it nearer to the posterior head by increments of 2.5 cm. The error due to distance from either head was up to 300% on taking the radioactivity counts from one head, and less than 8% on taking the sum of radioactivity counts from both heads (Fig. 2).

**Validation studies in vivo**

(a) In order to validate the Tc-HIDA radioactivity count in vivo, in six subjects the total abdominal radioactivity count of Tc-HIDA in vivo was compared with that of an identical dose of Tc-HIDA in vitro, obtained by using the phantom GB and maintaining the distance of the small container from the anterior scanner head approximately the same as that of the GB in vivo (checked by ultrasound). There was a very good correlation between the radioactivity counts obtained in vivo and in vitro (r = 0.993; P < 0.001).
(b) The total abdominal Tc-HIDA radioactivity counts in 14 subjects were measured on two separate occasions: in the fasting state, when a high proportion of the radioisotopic label is liable to be concentrated in a small area (GB) that is nearest to the anterior scanner head, and again 60 min after GB contraction induced by a Lundh test meal had caused GB contents to be distributed over a larger area and a variable depth. The total abdominal radioactivity counts, after correcting for radioisotope decay (99mTc half-life 6 h), correlated well for both measurements ($r = 0.99; P < 0.001$).

(c) In five subjects fasting-state GB filling was repeated on a separate occasion. Duplicate measurements of GB filling in the five subjects gave differences that varied between 1 and 8% (95% confidence limit 11%).

Further validation could be obtained by comparing our method of measuring biliary lipid mass with the results obtained after direct aspiration of GB contents at operation, but we have not done this.

**Application to healthy subjects**

Results for our 12 healthy volunteers are presented in Table 1. GB filling over 90 min ranged between 13 and 91% (mean $\pm$ SEM = 54.1 $\pm$ 7.5%). Mean SI of fasting-state GB bile was 0.93 $\pm$ 0.07 in 12 subjects in whom it was measured, and there was a significant correlation between GB filling and SI of fasting-state GB bile ($r = 0.63; P < 0.05$) (Fig. 3).

**TABLE 1. Full data for individual subjects used in the present study**

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Gallbladder filling (%)</th>
<th>SI</th>
<th>Bile acid pool size (mmol/l)</th>
<th>Biliary lipid mass in GB (mmol)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BA</td>
</tr>
<tr>
<td>1</td>
<td>67</td>
<td>1.07</td>
<td>8.1</td>
<td>5.2</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>1.08</td>
<td>11.8</td>
<td>8.7</td>
</tr>
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<td>3</td>
<td>24</td>
<td>0.79</td>
<td>10.1</td>
<td>6.6</td>
</tr>
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<td>4</td>
<td>71</td>
<td>0.90</td>
<td>6.5</td>
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</tr>
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<td>5</td>
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<td>0.72</td>
<td>7.5</td>
<td>3.8</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>1.06</td>
<td>9.0</td>
<td>4.6</td>
</tr>
<tr>
<td>7</td>
<td>91</td>
<td>0.84</td>
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<tr>
<td>8</td>
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<td>0.84</td>
<td>4.7</td>
<td>3.7</td>
</tr>
<tr>
<td>9</td>
<td>84</td>
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<td>5.4</td>
</tr>
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<td>13</td>
<td>0.55</td>
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<td>0.76</td>
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<tr>
<td>12</td>
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<td>–</td>
<td>4.1</td>
</tr>
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</table>

Mean $\pm$ SEM 54.1 $\pm$ 7.5 0.93 $\pm$ 0.07 7.6 $\pm$ 0.9 4.9 $\pm$ 0.5 1.6 $\pm$ 0.25 0.47 $\pm$ 0.09 29.9 $\pm$ 3.4
GB was 29.9 ± 3.4 ml. BA mass was 4.93 ± 0.51 mmol, phospholipid mass was 1.6 ± 0.25 mmol and cholesterol mass was 0.47 ± 0.09 mmol (Table 1). There was a significant correlation between GB volume and BA mass in GB \((r = 0.79; P < 0.005)\) but not between GB volume and total BA pool. There was also a significant correlation between total BA pool size and BA mass in GB \((r = 0.88; P < 0.001)\). Total BA pool size was 7.55 ± 0.9 mmol. The proportion of the BA pool in the GB in the fasting state in 11 subjects was 67 ± 5% (Table 1).

There was a significant correlation between SI of GB bile and mass of cholesterol in GB \((r = 0.64; P < 0.05)\) (Fig. 4). There was no significant correlation between SI of GB bile and GB mass of either BA or phospholipid \((r = 0.48\) and \(r = 0.04\) respectively).

Mean values for the Tc-HIDA radioactivity/BA concentration ratio in three successive GB bile samples from each of eight subjects were 6.6 ± 1.1, 7.4 ± 1.6 and 6.5 ± 1.0 c.p.m. x 1000 per mmol of BA. The corresponding mean values for SI were 1.05 ± 0.07, 1.05 ± 0.06 and 1.03 ± 1.0 (Fig. 5). There was no significant difference between the successive portions for either of these measurements. There was, however, a significant difference for the mean BA concentration between the first, second and third portions \((32, 37\) and 21 mmol/l respectively; \(P < 0.05)\).

BA concentration in the GB (derived from BA mass and GB volume) was 171 ± 27 mmol/l, phospholipid concentration was 54 ± 11 mmol/l, and cholesterol concentration was 15.7 ± 4.3 mmol/l.

**Discussion**

**GB filling**

O'Brien *et al.* [20] have demonstrated in the baboon that one-third to one-half of the overnight biliary lipid secretion enters the GB. Incomplete overnight GB filling has been demonstrated in man by Mok *et al.* [21] by a duodenal perfusion technique with bilirubin as a biliary marker. In another duodenal perfusion study, with indocyanine green as a bile marker, van Berge Henegouwen & Hofmann [22] found in a group of five young healthy volunteers a mean GB storage of 58% in the fasting state during the hour immediately before the morning meal. Our scintiscanning technique demonstrated similar GB filling (55% in a group of 12 young healthy volunteers), but our technique has the advantage of being non-invasive, simpler and more rapid, a complete study taking 90 min. It is therefore more conveniently applicable to a large number of subjects. In our 12 healthy volunteers, we found a significant correlation between GB filling and SI of fasting-state GB bile, suggesting that in health sequestration of supersaturated bile into the GB is a factor influencing SI of fasting-state GB bile.

A similar scintiscanning technique, published since the start of our study by Shaffer *et al.* [23], showed an average partitioning of fasting-state hepatic bile into the GB of 70% in healthy volunteers. This value may be falsely high, because those authors used an anterior scanning head only, and the GB usually lies nearer to the abdominal wall than does the gut. We have demonstrated in our
validation studies in vitro and in vivo that the error in radioactivity counting due to distance from the scanning head could be up to 300% when one head is used, as in their study, but only 8% with the use of two isosensitive scanning heads (placed anteriorly and posteriorly), as in our present study.

**Biliary lipid mass in GB**

By combining our scintiscanning technique with nasoduodenal intubation and cholecystokinin infusion to obtain a sample of fasting-state GB bile, we were able to measure the GB mass of all three biliary lipids for the first time. With their duodenal perfusion technique, Mok and colleagues [24] were able to determine the mass of bile acids in the GB, but not that of phospholipid and cholesterol, because they infused a liquid formula meal containing phospholipid and cholesterol. Our measurements, made with a much simpler technique, showed that the SI of fasting-state GB bile was significantly correlated with cholesterol mass in the GB ($r = 0.62; P < 0.05$), but not with bile acid and phospholipid mass, implying that cholesterol mass is the main determinant of SI in non-obese healthy males.

**Mixing of GB contents**

Nakayama & van der Linden [25] analysed GB contents obtained during surgery from 11 patients with gallstones in a functioning GB, and reported a stratification effect for BA concentration and SI, although the differences reported were not statistically significant. Our results do not confirm the presence of stratification in healthy subjects without GB disease. The previous findings could be due to cholesterol crystals detached from gallstones, causing a higher SI in the zone immediately around the stone than in other parts of the GB. Tera [14] has reported a stratification effect in healthy subjects for BA concentration. We confirmed this finding, but showed that the concentrations of other biliary lipids varied in parallel, so that SI remained constant. Tc-HIDA radioactivity counts also varied in parallel, so that the Tc-HIDA radioactivity/BA concentration ratio remained constant. Thus there are differences in dilution of all GB contents with water, presumably due to water absorption by the GB mucosa, but mixing of GB contents with each other is good.

**GB volume and concentrating power**

The method that we used for measuring GB volume by ultrasound examination has been previously validated by Everson et al. [15]. We found that GB volume was significantly correlated with BA mass in the GB ($r = 0.60; P < 0.01$) but not with total BA pool.

By combining GB volume with our measurements of biliary mass in the GB, we were able to assess the concentration of the bile acids and other substances within the GB. Previously this has only been possible by direct GB puncture at operation, since samples obtained by nasoduodenal intubation and cholecystokinin infusion are diluted to an unknown extent with pancreatic and other secretions. We obtained values of $171 \pm 27 \text{ mmol/l}$ for BA concentration, $54 \pm 11 \text{ mmol/l}$ for phospholipid and $15.7 \pm 4.3 \text{ mmol/l}$ for cholesterol concentration. These are similar values to those obtained after direct GB puncture at operation in normal controls by Nakayama & van der Linden [5, 26].

**Acknowledgments**

We are thankful to Mr Robert Bird for technical assistance and to Miss Marion Amos for secretarial assistance.

**References**


