precipitation of cholesterol crystals from cholesterol supersaturated bile is a key event in the formation of cholesterol gallstones. Other factors such as rapid nucleation and impaired postprandial gallbladder emptying have also been recognized as a risk factor for stone formation [1, 2]. Decreased gallbladder emptying and bile stasis may provide the residence time necessary for nucleation of cholesterol crystals and their subsequent acculation into macroscopic gallstones. The regulation of gallbladder motor activity involves complex interactions, still largely unknown, between neural and hormonal factors [3]. It is well established that the proximal gut hormone cholecystokinin is the hormonal mediator of gallbladder contraction in the intestinal phase [4]. Similarly, gastrin promotes gallbladder contraction [5, 6] whereas pancreatic polypeptide (PP) [7] and somatostatin [8, 9] have been shown as peptides enhancing gallbladder relaxation. Any alteration of the secretion of one or several hormones that lead to a decrease in gallbladder emptying, possibly because of gastrin activity as well as those of PP and somatostatin [13] arises whether endogenous hormone levels are altered in gallstone patients. Several studies found smaller fractional gallbladder emptying in patients with gallstones than in healthy controls [10, 11]. Others [12, 13] showed that endogenous CCK response in lithiasic patients following fat administration was impaired. In contrast, little is known concerning the defects of gastrin activity as well as those of PP and somatostatin [13] in lithiasis patients. Lastly, for some authors [14, 15], bile salts lead to a decrease in gallbladder emptying, possibly because gallbladder smooth muscle contraction is inhibited by bile salts.

The aim of the present study was to determine the characteristics of gallbladder emptying together with, gastrin, PP and somatostatin endogenous levels, and serum bile salt concentrations, before and after a fatty meal in gallstone patients compared with healthy subjects.

Materials and methods

Patients

Informed consent, in accordance with the Helsinki criteria, was obtained from each volunteer before enrolment in this study. Gallstone
patients and healthy subjects were comparable for gender, age, and body mass index (BMI) according to Quetelet's index (kg/m²). Two groups of subjects were considered for this study. Group 1 included 10 gallstone patients: 6 women and 4 men, median age 57 years (range: 35-85). All patients had radiolucent stones, 6 had solitary stones and 4 had more than 1 stone. None of these patients had clinical or laboratory signs of biliary complication in the 4 weeks before entering the study. Group 2 contained 20 subjects without lithiasis, 13 women and 7 men, median age 55 years (range: 30-82). No subject had a history of disease or surgery known to affect gallbladder motility, nor had taken any medication that could influence gallbladder contractility. No subject was diabetic, since it is known PP-like immunoreactive substance may occur in patients with diabetes mellitus [16].

**Gallbladder emptying**

Size and number of calculi were estimated during ultrasound. Gallbladder volume was calculated from longitudinal and cross sectional images according to the “sum-of-cylinders” method [17]. In patients with cholelithiasis, the calculation of gallbladder volume was performed after subtracting the stones’ volume. Gallbladder motility was assessed in each subject as follows: after an overnight fast, gallbladder volume was measured three times at 5 minutes intervals. A standard liquid test meal (Bladex®), consisting of 55 g of egg yolk, 25 g of saccharose, 10 g of rum, and 100 g of glycerin was administered. Gallbladder images were obtained for an additional 60 minutes at 10 minutes intervals. Results were expressed as fasting volume in milliliters (FV), measured immediately before the administration of the test meal, and residual volume in milliliters (RV), defined as the smallest post-meal residual volume. The ejection fraction (EF) was calculated according to the formula: EF = [(FV-RV)/FV] x 100 [18].

**Measurement of Plasma Hormones**

Blood samples for hormone measurements were obtained before, and 60 minutes after ingestion of the Bladex meal. For each subject, blood samples were taken between 8 and 10 AM. They were collected on EDTA (1 mg/mL) for gastrin, and on EDTA and Aprotinin (500 KIU/mL) for PP and somatostatin, and placed on ice. After centrifugation, plasma was frozen. A one milliliter sample was extracted on a column for chro-
matography C18 Sep (Amer sham, Sac lay, France). A competitive assay with I-125 labeled peptide was used for quantification. The following peptides were used: RIK 8004 somatostatin 28, and RIK 7198 human PP , from Peninsula Laboratories, inc. Belmont, CA. For gastrin assay, GASK-PP was from CIS Bio International, Gif-sur-Yvette, France. Measure-
ments were performed in duplicate with 100 µl aliquots.

The following hormonal parameters were evaluated: basal levels and hormone levels at 60 minutes. Serum bile salt concentrations were measured before and after 60 minutes following the fatty meal by using 3α-hydroxysteroid dehydrogenase (Enzabile, Nyegaard, Oslo, Norway).

**Statistical Analysis**

Data are expressed as medians and ranges. Statistical differences between groups were assessed by the Mann-Whitney test. Significance level was set at P < 0.05.

**Results**

**Analysis of gallbladder contraction**

Table I shows the characteristics of gallbladder emptying in 10 patients with gallstones and 20 healthy subjects. The fasting gallbladder volume tended to be larger in patients with cholelithiasis than in healthy subjects (median: 23.5 mL vs 20.0 mL; P < 0.08). The residual volume was about 2-fold higher in gall-
stone patients than in normal subjects. This difference was significant (P = 0.01). Patients with gallstones had a lower gall-
brad volume than healthy subjects (43% compared with 70%, P = 0.02). In lithiasis patients the ejection fraction tended to be larger in those with solitary stones as compared to multiple stones, but the difference did not reach statistical significa-
cence (data not shown).

**Hormone levels**

As shown in table II, fasting PP concentrations were signifi-
cantly higher in the lithiasis group (median: 51 pg/mL, range 22-123) than in controls (median: 32 pg/mL, range 17-92) (P < 0.03). After ingestion of the fatty meal, all subjects had increased hormone levels (P < 0.01). Postprandial levels were similar in the two groups. The difference observed between the postprandial and the basal values might be considered as an index of hormone release. This index is lower in patients with lithiasis than in healthy subjects although it failed to reach significa-
cence for the non-parametric test used (P = 0.07). There was a significant correlation between fasting PP concentrations and residual gallbladder volume in patients with lithiasis (r = 0.70; P < 0.03) and in controls (r = 0.58; P < 0.01).

For somatostatin, no differences were observed between the two groups of subjects neither in basal levels nor in postprandial concentrations (table II).

Basal gastrin levels were nearly identical in the two groups. After the fatty meal, no difference was found between lithiasis patients and controls (table II).

**Serum bile salt levels**

Fasting serum bile salt concentrations of patients with lithiasis and healthy subjects were similar. Median values were 3.8 µM and 2.6 µM respectively (P = 0.06). No difference was observed in post-prandial bile salt levels between the two groups (table II).

**Discussion**

Data from the present study indicate that fasting plasma PP concentrations are significantly higher in patients with cholelithi-

asia than in healthy controls. Moreover, in lithiasis patients, reduced gallbladder contractility is shown by an increased resid-
ual volume and by a lower ejection fraction.

The gallbladder is not a mere storage sack for bile that em-
pies postprandially and refills between meals. Hepatic bile flow enters the gallbladder only in part [2, 21]. There seems to be very frequent short periods of gallbladder emptying and refilling that occurs not only postprandially but also during fasting, thus con-
tributing to mixing of gallbladder contents [22]. These dynamics are crucial to ensure the turnover of gallbladder bile and preclude biliary stasis that might promote cholesterol crystal precipitation

<table>
<thead>
<tr>
<th><strong>Table I.</strong> Characteristics of gallbladder emptying in patients with cholelithiasis and in control subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with gallstones</strong></td>
</tr>
<tr>
<td>N = 10</td>
</tr>
<tr>
<td><strong>Fasting volume (mL)</strong></td>
</tr>
<tr>
<td><strong>Residual volume (mL)</strong></td>
</tr>
<tr>
<td><strong>Ejection fraction (%)</strong></td>
</tr>
</tbody>
</table>

Data are expressed as medians [ranges]
Table II. - Basal and postprandial (at 60 min) plasma concentrations of gut hormones and bile salts in patients with cholelithiasis and in control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Patients with gallstones N = 10</th>
<th>Control subjects N = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic polypeptide (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>51.0 [22.0-123.0]</td>
<td>32.0 [17.0-92.0]</td>
</tr>
<tr>
<td>Postprandial</td>
<td>74.0 [61.0-277.0]</td>
<td>99.0 [44.0-181.0]</td>
</tr>
<tr>
<td>Somatostatin (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>10.0 [1.9-20.0]</td>
<td>10.5 [3.7-22.0]</td>
</tr>
<tr>
<td>Postprandial</td>
<td>13.5 [1.9-33.0]</td>
<td>19.0 [3.9-29.0]</td>
</tr>
<tr>
<td>Gastrin (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>41.5 [6.0-50.0]</td>
<td>41.0 [29.0-50.0]</td>
</tr>
<tr>
<td>Postprandial</td>
<td>46.0 [36.0-174.0]</td>
<td>44.0 [35.0-174.0]</td>
</tr>
<tr>
<td>Bile salts (μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.8 [2.0-6.7]</td>
<td>2.6 [1.5-6.2]</td>
</tr>
<tr>
<td>Postprandial</td>
<td>5.0 [2.0-20.0]</td>
<td>4.2 [1.5-10.2]</td>
</tr>
</tbody>
</table>

Data are expressed as medians [ranges]

* Differs from controls (P < 0.03)

and stone formation. During fasting, the plasma concentration of cholecystokinin, the antagonist hormone of PP, is minimum, thus allowing for a maximal relaxing effect of PP on the gallbladder wall. Our data indicate that during the interdigestive period, the mean plasma PP level in lithiasis patients is higher (+60%) than in healthy subjects while the gallbladder volume is larger (+25%). These findings are consistent with a relationship between the plasma level of PP and the relaxation of the gallbladder. By contrast, such a relationship is not found in the postprandial period.

References


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REFERENCES


