Gallbladder motility and gut hormone plasma levels in subjects with and without gallstones

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SUMMARY

Hormonal control of gallbladder motility is still unclear in patients with cholelithiasis. In a case-control study, we determined the characteristics of gallbladder emptying evaluated sonographically and the hormone levels of somatostatin, gastrin, and pancreatic polypeptide, before and after a fatty meal in 10 gallstone patients compared with 20 healthy subjects. Patients with lithiasis had a larger residual volume (median 12.0 ml vs 6.5 ml; P = 0.01) and a lower gallbladder ejection fraction (43% vs 70%; P = 0.02) than healthy subjects. During fasting, plasma pancreatic polypeptide concentrations were significantly higher in lithiasis patients (P < 0.03). In contrast, no differences between the two groups of patients were observed during the post prandial period. Somatostatin and gastrin plasma levels were similar in the two groups. Lastly, the serum bile salt levels were in the normal range and were not different between groups both during fasting and postprandial states. We conclude that large basal plasma concentrations of pancreatic polypeptide, a gut peptide inducing gallbladder relaxation, may constitute a factor facilitating lithogenesis.

RÉSUMÉ

Motilité de la vésicule biliaire et taux circulants des hormones gastro-intestinales chez des sujets avec et sans calculs biliaires

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Le contrôle hormonal de la motricité vésiculaire chez les malades lithiasiques n’est pas clairement établi. Dans une étude cas-témoins nous avons déterminé les caractéristiques de la motricité vésiculaire par échographie ainsi que les taux plasmatiques de somatostatine, de gastrine, et du polypeptide pancréatique, avant et après un repas gras, chez 10 malades lithiasiques et 20 sujets contrôles. Les malades lithiasiques avaient un plus grand volume résiduel (médiane : 12.0 ml vs 6.5 ml ; P = 0.01) et une plus petite fraction d’éjection vésiculaire (43 % vs 70 %, P = 0.02) que les sujets témoins. Par ailleurs, les concentrations du polypeptide pancréatique étaient plus élevées chez les malades lithiasiques que chez les témoins (P = 0.03) au cours du jeûne, alors qu’aucune différence n’était observée en période postprandiale. Les taux plasmatiques de somatostatine et de gastrine étaient semblables dans les deux groupes. Les taux sériques de sels biliaires étaient normaux dans les deux groupes, aussi bien à jeun qu’après le repas gras. En conclusion, pendant la période de jeûne, des concentrations élevées de polypeptide pancréatique pourraient jouer un rôle favorisant la lithogénèse.

P 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 accululation 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 may induce an increase in gallbladder hypomotility will play a facilitating role in cholesterol precipitation. Thus, the question 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 choledolithiasis, the calculation of gallbladder volume was performed after substracting 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: \( EF = \left( \frac{FV - RV}{FV} \right) \times 100 \) [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 chromatography C18 Sep (Amersham, Saclay, France). A competitive assay with 1-125 labeled peptide was used for quantification. The following peptides were used: RIK 8004 somatostatin 28, and RIK 7198 human PP, from GASK-PR was from CIS Bio International, Gif-sur-Yvette, France. Measurements were performed in duplicate with 100 µl aliquots.

The following hormonal parameters were evaluated: basal levels and hormone levels at 60 minutes. The time for blood sampling was selected in order to avoid variability in gastric emptying, and because it was demonstrated that plasma levels of gut hormones reached a plateau value at 60-90 minutes after meal ingestion [19, 20].

**Serum bile salt analysis**

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 choledolithiasis than in healthy subjects (median: 23.5 mL vs 20.0 mL; \( P < 0.08 \)). The residual volume was about 2-fold higher in gallstone patients than in normal subjects. This difference was significant (\( P = 0.01 \)). Patients with gallstones had a lower gallbladder ejection fraction 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 significance (data not shown).

**Hormone levels**

As shown in table II, fasting PP concentrations were significantly 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 significance 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).

**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 postprandial 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 choledolithiasis than in healthy controls. Moreover, in lithiasis patients, reduced gallbladder contractility is shown by an increased residual volume and by a lower ejection fraction.

The gallbladder is not a mere storage sack for bile that empties 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 contributing 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 I** – Characteristics of gallbladder emptying in patients with choledolithiasis and in control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Patients with gallstones</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting volume (mL)</td>
<td>23.5 [14-38]</td>
<td>20.0 [11-28]</td>
</tr>
<tr>
<td>Residual volume (mL)</td>
<td>12.0 [7-27]</td>
<td>6.5 [2-15]</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>43.5 [14-67]</td>
<td>70.0 [24-88]</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 (nM)</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. An impaired gallbladder emptying in patients with gallstones, characterized by a lower gallbladder ejection fraction (-30%) and a larger residual volume (+86%) is observed for similar PP plasma concentrations in the two groups of patients.

It appears that the fasting state brings together a set of conditions favourable to crystallization of cholesterol. Indeed, the gallbladder motor defects we observed in patients with cholelithiasis, occur when bile is supersaturated with cholesterol due to normal diurnal variation in biliary lipid composition. During overnight fasting, the observed cholesterol/phospholipid ratio is maximum [23]. The pathogenesis of cholesterol gallstones is a multifactorial process, including supersaturation of bile with cholesterol, precipitation of cholesterol crystals, and gallbladder hypomotility permitting crystal growth, agglomeration and retention of microstones which then grow to macroscopic gallstones [2]. Therefore, PP by enhancing gallbladder relaxation may have an important role on lithogenesis in modulating gallbladder contractility dynamics.

On the other hand, postprandial refilling and turnover are specific defects in patients with gallstones [21]. In healthy subjects, a large part of hepatic bile enters the gallbladder before reaching the duodenum. The cholecystoduodenal division of hepatic bile changes from 50/50 in the healthy state to 20/80 in favour of the small intestine in the lithogenic state [2, 21]. There is a very marked difference between lithiasis patients and healthy subjects in the volume of bile turned over in the gallbladder and consequently, the washout effect is drastically decreased in gallstone patients. Impaired mixing of the gallbladder content induces stasis, thus facilitating cholesterol crystal precipitation. The large plasma PP concentration we observed during fasting constitutes an additional factor for lithogenesis.

Our data failed to show a difference in PP levels between the two groups during the postprandial period. This is in accordance with Glasbrenner et al. [13] who showed that postprandial plasma concentrations of endogenous PP were similar in patients and controls.

In patients with gallstones, between meals, about 80% of hepatic bile enters the small intestine. On the basis of our results, we may postulate that the enhancement in the enterohepatic cycling of bile salts in these patients does not lead to an increased bile salt plasma concentration, probably because of the efficient hepatic uptake of bile salts. Indeed, no statistical differences in the basal bile salt levels were observed between lithiasis patients and control subjects. In contrast, we cannot exclude that the increased flux of bile salts in the intestinal lumen in patients with lithiasis during the interdigestive state may induce an increase in PP serum levels.

Basal somatostatin plasma levels we observed were similar in both groups. Also, no difference was seen after the fatty meal. It is noteworthy that the somatostatin plasma levels we obtained are many times lower than those obtained by Fischer et al. [8] who found an inhibition of gallbladder emptying response to test meals following somatostatin infusion (7 µg/kg.h) in normal volunteers. Similarly, large doses of the somatostatin analogue given to acromegalic patients, impaired gallbladder emptying and probably explain the high incidence of gallstones in these patients [24]. In our protocol, the endogenous variations of somatostatin levels do not appear to play a role in the pathogenesis of cholelithiasis.

In conclusion, the present study shows that, in patients with lithiasis, gallbladder hypomotility is associated with high fasting plasma concentrations of PP, which therefore may contribute to the development of cholesterol gallstone disease. In contrast, we found no evidence for a pathogenic role of circulating somatostatin, gastrin and bile salts.

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REFERENCES


