Relationship between hepatic cholesterol synthesis and biliary cholesterol secretion in man: hepatic cholesterol synthesis is not a major regulator of biliary lipid secretion

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Summary

1. To examine the role of newly synthesized cholesterol as a determinant of bile lipid secretion, both hepatic cholesterol synthesis (as judged by the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, EC 1.1.1.34; HMGCoAR) and steady state biliary cholesterol output were measured in nine patients.

2. HMGCoAR levels varied four fold (9-40 pmol min$^{-1}$ mg$^{-1}$) and biliary cholesterol secretion 2.5-fold (0.60-1.15 pmol h$^{-1}$ kg$^{-1}$) but there was no correlation between these two variables ($r = 0.18; P > 0.05$) nor between biliary bile acid output and HMGCoAR activity ($r = 0.34; P > 0.05$).

3. There was, however, a linear relationship between bile acid and phospholipid secretion ($r = 0.77; P < 0.001$) and between bile acid and cholesterol secretion ($r = 0.69; P < 0.05$).

4. These results suggest that HMGCoAR activity is not a major determinant of cholesterol secretion and at these secretion rates is HMGCoAR activity related to bile acid return to the liver.

Key words: bile acid and salt secretion, cholelithiasis, cholesterol.

Abbreviation: HMGCoAR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

Introduction

The contribution of hepatic cholesterol synthesis to biliary cholesterol secretion is disputed, partly because techniques to measure bile lipid secretion in intact (non-operated) subjects have only become available recently [1] and partly because there is relatively little information about hepatic cholesterologenesis in man [2-5]. Furthermore, the limited data available provide conflicting information. In one study of 11 gallstone patients, Key et al. [6] found that there was a significant linear relationship between hepatic cholesterologenesis (as judged by the activity of the rate limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGCoAR) and biliary cholesterol secretion. They suggested therefore that newly synthesized cholesterol was a major determinant of biliary cholesterol secretion. However, using entirely different techniques (computer analysis of specific radioactivity decay curves in blood and bile after pulse labelling of cholesterol and its precursors), Schwartz et al. [7] suggested that only 30% of biliary cholesterol was newly synthesized.

The aims of the present investigation, therefore, were: (i) to study further, steady-state bile lipid secretion and hepatic HMGCoAR activity in a group of patients who were selected in anticipation that they would have a wide scatter of results for both variables (and therefore the opportunity to see whether or not the variables were related), and thus in contrast to Key et al. [6] we chose patients with and without gallstones; (ii) to see if HMGCoAR activity was related to the biliary cholesterol saturation in these same patients (since gallstone patients have an absolute or relative increase in the cholesterol content of bile [8, 9] and greater than normal HMGCoAR.
activities [2, 3]) and (iii) to see whether there was an inverse relationship between hepatic cholesterol synthesis and biliary bile acid secretion, because certain animal studies suggested such a relationship existed [10]. This paper reports our findings; the work was presented in part at the Medical Research Society meeting, London and published in abstract form [11].

Methods

Study design

Nine patients, in whom elective laparotomy had been planned for vagotomy or cholecystectomy, or for the investigation of abdominal pain, consented to take part in this study. These included patients with radiolucent, presumed cholesterol-rich gallstones, patients with radiopaque, pigment-rich stones and patients who were free of gallstones. Clinical details of the nine patients are given in Table 1. Apart from the presence of gallstones, all were clinically well. None had hyperlipidaemia or diabetes mellitus and none was taking medication at the time of surgery. In all nine patients the bile lipid secretion studies were carried out on the day before surgery and at laparotomy the following day a wedge biopsy of liver was obtained between 10.30 and 12.00 hours. The studies were approved by the Ethics Committee of Guy's Hospital and Medical School.

Laboratory methods

Bile lipid secretion rates were measured by a perfusion method modified from that of Shaffer & Small [9]. After the patient had fasted overnight, a three-lumen radio-opaque tube was positioned in the duodenum under radiographic control such that the infusion and proximal aspiration ports lay at the level of the ampulla of Vater and the distal port lay 15 cm downstream. A phosphate-free amino acid solution (Vamin-N, KabiVitrum, Sweden), diluted 1:3 with distilled water, supplemented with methionine (22 mmol/l), valine (52 mmol/l) and phenylalanine (22 mmol/l) and containing bromosulphthalein (BSP; 100 mg/l) as a poorly-absorbable marker was infused at a rate of 2 ml/min, giving delivery rates of 26-0 mmol/h for methionine, 76-8 for for valine and 40-2 for phenylalanine, rates known to stimulate pancreatic secretion maximally (and, by implication, to stimulate cholecystokinin release and gallbladder contraction [12]). When measured by freezing-point depression, the infusate was nearly iso-osmolar (300–320 mosmol/kg).

After a 4 h equilibration period, duodenal contents were aspirated in hourly portions of 6 ml for 6 h from both the proximal and distal aspiration points. The concentrations of bile acids, phospholipids [13] and cholesterol [14] in the proximal aspirate, of cholesterol in the distal aspirate, and of BSP [15] in both the infusate and in the distal aspirate, were then estimated and secretion rates of the three biliary lipids calculated from standard equations [9].

Hepatic HMGCoAR activity

Liver biopsies were rapidly homogenized, a microsomal fraction was prepared and enzyme activity determined as previously described [3]. Results were expressed as pmol of mevalonate formed min⁻¹ mg⁻¹ of microsomal protein.

Gallstone analysis

The gallstones obtained at operation were dried to constant weight, crushed, extracted into propan-2-ol and their cholesterol contents determined [14].

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Ideal body wt. (%)</th>
<th>Cholesterol secretion (μmol h⁻¹ kg⁻¹)</th>
<th>Phospholipid secretion (μmol h⁻¹ kg⁻¹)</th>
<th>Bile acid secretion (μmol h⁻¹ kg⁻¹)</th>
<th>Stone cholesterol (%)</th>
<th>HMGCoAR activity (pmol min⁻¹ mg⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>62.6</td>
<td>100</td>
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<td>11.60</td>
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<td>—</td>
<td>12.4</td>
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<tr>
<td>2</td>
<td>M</td>
<td>74.1</td>
<td>108</td>
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<td>3.54</td>
<td>6.56</td>
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<td>0.63</td>
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<td>—</td>
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<tr>
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<td>3.82</td>
<td>14.40</td>
<td>91.4</td>
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</tbody>
</table>
Statistics

Standard linear regression analyses were used to see whether or not there were significant correlations between the variables. For correlations between HMGCoAR and biliary lipid secretion mean lipid secretion values were used but for correlations between secretion of various lipids, hour by hour secretion values were used to provide more data points.

Results

Bile lipid secretion

The mean hourly steady state bile acid phospholipid and cholesterol secretion rates for the nine patients are shown in Table 1. Bile acid secretion varied six fold from 7 to 42 μmol h⁻¹ kg⁻¹ and cholesterol secretion 2.5-fold from 0.60 to 1.15 μmol h⁻¹ kg⁻¹.

HMGCoAR activity

The results of HMGCoAR activity are also shown in Table 1. The enzyme activity varied between 9 and 40 pmol min⁻¹ mg⁻¹ of microsomal protein.

Relationships between individual bile lipid secretion rates and between bile lipid secretion and HMGCoAR activity

There was a significant linear relationship between hourly secretion rates for bile acids and phospholipids \((r = 0.77; P < 0.001)\). There was also a significant linear relationship between bile acid and cholesterol secretion \((r = 0.69; P < 0.001)\). However, there were no correlations between HMGCoAR activity and biliary cholesterol secretion \((r = 0.18; P > 0.05)\), nor between the enzyme activity and the cholesterol content (mol % cholesterol) in bile \((r = 0.18; P > 0.05)\). No inverse or other relationship was noted between HMGCoAR and bile acid secretion \((r = 0.34; P > 0.05)\) and since most of the bile acid secretion derives from recycled bile acids, this implies that bile acid return to the liver does not affect HMGCoAR activity.

Gallstone analyses

Results of the gallstone analyses are shown in Table 1. Two of the four patients with radiolucent gallstones had cholesterol-rich (>80% cholesterol by weight) stones and two had mixed (40–60% cholesterol) stones. The radio-opaque stones were pigment-rich and contained only small amounts (<10%) of cholesterol.

Discussion

In this study, a small group of patients with a relatively wide range of hepatic cholesterol synthesis rates was used to examine the relationship between hepatic cholesterol synthesis and biliary lipid secretion. The results in these patients suggest that hepatic HMGCoAR (and therefore newly synthesized cholesterol) is not a major determinant of biliary cholesterol secretion and that bile acid secretion does not affect HMGCoAR.

The validity of these conclusions depends on the reliability of the methods. Although the use of a continuous amino acid infusion to simulate bile lipid secretion seems unphysiological, nonetheless it gives similar results to those obtained by the relatively more physiological method of perfusing three liquid meals after an overnight fast [16]. Furthermore, as only small portions of duodenal contents were removed, less than 10% of the secreted bile acids were aspirated and therefore the enterohepatic circulation of bile acids remained virtually intact [17]. Indeed, the pattern of results for bile lipid secretion in the present report is similar to that found in other studies where an even wider range of bile lipid secretion rates than is reported here was induced, either by bile acid feeding or by depletion of the bile acid pool [18–20].

Measurement of HMGCoAR activity is only one method of estimating hepatic cholesterol synthesis, but the results obtained with this technique yield qualitatively similar results to those obtained by other methods [21–22]. Extrahepatic cholesterogenesis was not measured in the present study and although hepatic cholesterol synthesis was thought to account for most of the cholesterol synthesized in the body [23] the contribution of non-hepatic cholesterogenesis to overall cholesterol metabolism may well be more important than has previously been thought [24].

The presumption that cholesterol secretion measured on one day can with validity be correlated with reductase activity measured in an operative liver biopsy taken on the next, requires that both cholesterol secretion and synthesis are reproducible, and that surgery and the associated anaesthesia do not affect HMGCoAR activity.

There is evidence that biliary lipid secretion as measured by perfusion techniques is in fact reproducible [25] but there is no comparable evidence for reproducibility of hepatic HMGCoAR activity in man. However, in a steady state of cholesterol balance it seems likely that cholesterol synthesis (and by implication HMGCoAR activity) will remain constant.
Anaesthesia and surgical stress do not appear to affect hepatic HMGCoAR activity [26-27]. It should be emphasized, however, that patients were perfused on one day and had their liver biopsies on the next. The patients underwent an overnight fast both before the perfusion study (12 h) and before surgery (13-15 h) when the liver biopsy was taken. Thus the essential difference between the 2 study days was 1-3 h further of complete fast as opposed to a controlled perfusion of a cholesterol-free infusion providing only 30-90 kcal. Thus the difference in bile physiology between the perfusion day and the operative day is that of endogenous bile lipids entering the intestine for 1-3 h under the stimulus of the perfusion. Although in animal models the ingestion of both bile acids [28] and cholesterol [29] can directly or indirectly affect hepatic cholesterol synthesis, in our study the modest amounts of these endogenous lipids entering the intestine as a result of the perfusion are unlikely to have affected hepatic HMGCoAR activity. Furthermore HMGCoAR activity is modified principally by changes in the amount of enzyme protein present and since the period of perfusion was relatively short it seems unlikely that this would have changed markedly. Finally all patients underwent the same protocol and thus any factors affecting secretion or synthesis are likely to have been common to all. For all these reasons we believe that the comparisons between lipid secretion and hepatic HMGCoAR activity made in this paper are valid.

The lack of correlation between HMGCoAR activity and biliary cholesterol secretion supports the findings of Schwartz et al. [7]. Using radioisotopic techniques in post-cholecystectomy patients with common duct T-tubes, they found that only 30% of the cholesterol in bile came from a newly synthesized hepatic pool. Similar conclusions were reached by Long et al. [30], who used different radioisotopic methods in the rat, although Turley & Dietschy [31], who also studied the rat, were unable to find any relationship between biliary cholesterol secretion and synthesis over a 100-fold range in hepatic cholesterogenesis.

The present results differ from the initial report by Key et al. [6] showing that hepatic reductase activity was linearly related to biliary cholesterol secretion in a group of untreated gallstone patients, though we studied patients with and without gallstones. However, the same authors were unable to confirm this finding subsequently when they again studied the relationship between these variables in patients treated with chenodeoxycholic acid [32]. Indeed, a simple relationship between hepatic cholesterol synthesis and biliary cholesterol secretion would be surprising since, apart from direct secretion into bile, newly synthesized hepatic cholesterol could, in theory, act as a substrate for bile acid synthesis, could be incorporated into circulating lipoproteins or into intrinsic cellular structures. On the other hand, cholesterol secreted into bile could arise not only from newly synthesized hepatic cholesterol but also from dietary cholesterol or from extrahepatic sources.

In the present study there was no significant relationship between hepatic HMGCoAR activity and the 'physiological range' of biliary bile acid secretion. This contrasts with the pattern of results in experimental animals when the enterohepatic circulation was markedly disturbed either by creating a bile fistula, which increased [33] hepatic cholesterogenesis, or by bile acid feeding, which decreased [34] hepatic cholesterogenesis. However, the present results are in accord with the hypothesis, advanced by Nervi & Dietschy [35] that bile acids exert a major influence on cholesterogenesis only at high bile acid secretion rates or when the enterohepatic circulation is broken.

Given the fact that there is no correlation between hepatic HMGCoAR activity and biliary cholesterol secretion, one must question the patho-physiological significance of elevated reductase levels in untreated gallstone patients as a factor influencing bile lipid abnormalities.

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References


