Do colonic bacteria contribute to cholesterol gall-stone formation? Effects of lactulose on bile

J R THORNTON, K W HEATON

Abstract
Ten healthy middle-aged women volunteered for a study to test the effect of lactulose—a synthetic, non-absorbable disaccharide—on the colonic metabolism of bile acids and on bile lipid composition. Lactulose (60 g daily in eight cases, 39 g daily in two) was taken as a proprietary syrup for six weeks, and bile was collected by duodenal intubation before and immediately after the six weeks.

All subjects showed a fall in the percentage of the 7α-dehydroxylated bile acid deoxycholic acid (mean 28.4±SEM 3.7 to 15.6±2.4; p < 0.002) and a rise in the percentage of the primary bile acid chenodeoxycholic acid (mean 33.2±3.2 to 42.9±2.8; p < 0.001). The percentage of cholic acid rose in eight subjects but mean values did not differ significantly. Bile was initially supersaturated with cholesterol in most subjects and became less saturated with cholesterol in all but one (mean saturation index 1:40-0:11 to 1:19:0:07; p < 0.005).

These data support the theory that colonic bacteria contribute to cholesterol gall-stone formation.

Introduction
Bile acids which escape reabsorption in the terminal ileum pass into the colon. There, bacterial 7α-dehydroxylation converts primary bile acids into the secondary forms, cholic acid becoming deoxycholic acid, chenodeoxycholic acid becoming lithocholic acid. These secondary bile acids are partially absorbed and enter the bile acid pool. Feeding small amounts of deoxycholic acid reportedly raised bile cholesterol saturation, leading to the suggestion that increased return of deoxycholic acid to the liver alters its metabolism to favour the secretion of bile supersaturated with cholesterol and hence apt to precipitate cholesterol gall stones. Feeding larger amounts of deoxycholic acid, however, did not produce this effect. Administration of metronidazole, an antimicrobial agent active against many colonic bacteria, reduced the proportion of deoxycholic acid in bile, raised that of chenodeoxycholic acid, and lowered bile cholesterol saturation.

In vitro, 7α-dehydroxylation of bile acids is inhibited below pH 6·0-6·5. Lactulose, a synthetic, non-absorbable disaccharide, is metabolised to organic acids by the colonic flora and reduces the pH in the right colon to less than 5·0. We therefore predicted that lactulose would reduce 7α-dehydroxylation. To test this hypothesis and look for associated improvement in bile cholesterol saturation we have analysed bile before and after administration of lactulose.

Subjects and methods
Ten apparently healthy women aged 42-51 years (mean 46) volunteered for the study. Their mean body weight was 118% of ideal (range 104-13%). Six subjects were chosen as being known from other studies to have bile supersaturated with cholesterol. For ethical reasons oral cholecystograms were not performed, but no subject had symptoms of gall-bladder disease. As some subjects had highly supersaturated bile, however, a few may have had asymptomatic gall stones. Standard liver function values were normal, and no subject was taking any medication, including oral contraceptives. Subjects maintained their usual diet and activities throughout the study and kept a daily record of bowel movements.

The women were given a proprietary syrup containing 670 g lactulose, 60 g lactone, and 110 g galactose per l. They were instructed...
to take 15 ml three times daily with meals for one week and then to increase each dose to 30 ml, giving a daily dose of 60 g lactulose, for a further five weeks. Bile was collected by duodenal intubation before and immediately after the six weeks of lactulose administration. After an overnight fast duodenal contents were aspirated following gallbladder stimulation with cholecystokinin. This evoked a prompt flow of more-concentrated bile (total lipid concentration 18-98 mmol/l, median 54 mmol/l). Samples obtained in this way have a lipid composition which accurately reflects that of gall-bladder contents.19

Both bile samples from each subject were analysed in the same batch. Concentrations of total bile salts, phospholipids, and cholesterol18 were used to calculate a cholesterol saturation index using the criteria of Hegardt and Dam.19 The individual bile acid composition was determined by gas-liquid chromatography. The statistical significance of differences was calculated by Student’s t test.

### Results

After taking lactulose the mean percentage of deoxycholic acid in bile decreased from 28.4 \pm SEM 3.7 to 15.6 \pm 2.4 (p < 0.002). This fall occurred in all 10 subjects (fig 1). Conversely, all subjects showed a rise in the percentage of chenodeoxycholic acid (33.2 \pm 3.2 to 42.9 \pm 2.9; p < 0.001). The percentage of cholic acid rose in eight subjects but the mean values of 36.0 \pm 1.4 and 39.0 \pm 1.3 were not significantly different (fig 1). Lithocholic acid was present in trace amounts before and after lactulose (2.5 \pm 0.2 on both occasions).

Bile became less saturated with cholesterol in nine subjects (fig 2). Mean saturation index fell from 1.40 \pm 0.11 to 1.19 \pm 0.07 (p < 0.005).

The table shows the mean molar percentages of total bile acids, phospholipids, and cholesterol before and after lactulose.

<table>
<thead>
<tr>
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<th>Total bile acids</th>
<th>Phospholipids</th>
<th>Cholesterol</th>
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<tr>
<td>Before lactulose</td>
<td>74.1 \pm 1.3</td>
<td>18.1 \pm 1.0</td>
<td>7.8 \pm 0.8</td>
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<tr>
<td>After lactulose</td>
<td>76.0 \pm 1.0</td>
<td>17.5 \pm 0.5</td>
<td>6.5 \pm 0.5</td>
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<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.02</td>
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NS = Not significant.

### Discussion

This study shows that administration of lactulose can lower the cholesterol saturation of bile. The mechanism of the reduction is not disclosed by our data. It may be due to reduced cholesterol secretion or expansion of the bile acid pool, or both. Possibly it is linked with the increased proportion of biliary chenodeoxycholic acid, since administration of this bile acid lowers bile cholesterol saturation.18 The simplest explanation for the rise in chenodeoxycholic acid is improved intestinal conservation because of reduced 7α-dehydroxylation to lithocholic acid—a rapidly excreted bile acid. Nevertheless, the proportion of the other primary bile acid, cholic acid, did not rise significantly. Thus our results are also compatible with the suggestion14 that deoxycholic acid selectively inhibits the hepatic synthesis of chenodeoxycholic acid, reduced production or absorption of deoxycholic acid during lactulose administration allowing the liver to produce more chenodeoxycholic acid. As we measured percentages rather than pool sizes of the individual bile acids, however, the absolute amount of their change with administration of lactulose remains uncertain.

The reduction in deoxycholic acid seen in all subjects has several possible explanations. In view of its laxative properties lactulose may accelerate colonic transit, so reducing the time available for the formation or absorption of secondary bile acids. Alternatively, acidification of the colon may increase biliary acid excretion, either by precipitation15 or by promoting the binding of bile acids to lignin, a component of dietary fibre.16 The data, however, are consistent with our hypothesis that, by acidifying the colon, lactulose reduces bacterial 7α-dehydroxylation of bile acids.

The effects of lactulose on bile resemble those of wheat bran. Like lactulose, bran lowers biliary deoxycholic acid, increases chenodeoxycholic acid, and can lower cholesterol saturation.17 The mechanism of action of bran has not been elucidated. Nevertheless, bran is partly metabolised by colonic bacteria to short-chain fatty acids,18 and we suggest that colonic acidification and consequent reduction of bile acid 7α-dehydroxylation may partly explain its action on bile.

The clinical relevance of our findings is uncertain. To dissolve existing gall stones probably requires that saturation index be reduced to less than 1.019 This did not occur in this study. Nevertheless, a greater effect may, perhaps, be obtained with more-prolonged administration of lactulose. The relatively modest fall in saturation index seen in this study could be useful in reducing the risk of cholesterol gall-stone formation in high-risk groups or in reducing the possibility of recurrence of gall stones after dissolution by other means.

In conclusion, our findings support the concept that colonic bacterial metabolism of bile acids plays a part in the production of bile supersaturated with cholesterol and hence cholesterol gall-stone formation.

We thank Duphar Laboratories Ltd for providing lactulose syrup. Requests for reprints should be addressed to JRT.
Diagnosis of deep vein thrombosis using autologous indium-III-labelled platelets

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Abstract

Forty-eight patients who had undergone surgical reduction of a fractured neck of femur or in whom deep vein thrombosis was suspected clinically were studied by ascending phlebography and imaging after injection of autologous indium-111-labelled platelets to assess the accuracy and value of the radioisotopic technique in diagnosing deep vein thrombosis. Imaging was performed with a wide-field gammacamera linked with data display facilities. Phlebography showed thrombi in 26 out of 54 limbs examined and a thrombus in the inferior vena cava of one patient; imaging the labelled platelets showed the thrombi in 24 of the 26 limbs and the thrombus in the inferior vena cava.

The accumulation of indium-111 at sites corresponding to those at which venous thrombi have been shown phlebographically indicates that this radioisotopic technique is a useful addition to methods already available for the detection of deep vein thrombosis.

Introduction

The diagnosis of deep vein thrombosis on the basis of clinical signs is difficult and frequently incorrect, with the exception of phlegmasia dolens, when the clinical features are reliable indicators. Most deep vein thrombi, however, are less extensive and thus present fewer clear-cut clinical features than phlegmasia, though they still carry the risk of major pulmonary embolism. This risk necessitates objective confirmation of a clinical suspicion of deep vein thrombosis, and various methods have been used for this including ascending phlebography, detection of Doppler flow, impedance plethysmography, thermography, and radioisotopic techniques (111I-fibrinogen, 18F-fibrinogen, 99mTc-macroaggregated albumin, 99mTc-urokinase, and 99mTc-plasminogen). Unfortunately, all these methods have drawbacks limiting their use. An ideal diagnostic technique must be painless; non-invasive; safe and free from side effects; simple and reliable; rapid; accurate in detecting the site, size, and number of thrombi, whether recent or old; repeatable; financially acceptable; and require minimal transportation of the patient. No method currently satisfies these criteria, and new methods require to be established and examined.

We describe a simple, rapid technique for labelling autologous human platelets with indium-111 and imaging with a gammacamera to detect deep vein thrombosis and compare the accuracy of this method with that of ascending phlebography.

Materials, methods, and patients

Autologous platelets were labelled by using the technique developed by Hawker et al. This entailed removing 26 ml of blood and separating the platelets, which were labelled with 180-220 μCi of indium-111 oxine (Radiochemical Centre) and reinserted within 45 minutes of