

urine. Subsequently he became confused and disorientated. On examination his temperature was found to be 37.8°C and he was jaundiced and drowsy, with petechiae on arms, legs, and trunk. Haemoglobin concentration was 9.0 g/dl, white cell count $5.0 \times 10^9/l$, and platelets $< 10 \times 10^9/l$, and the blood film showed pronounced red-cell fragmentation. Urine analysis showed heavy proteinuria and haematuria. He was treated with fresh-frozen plasma, hydrocortisone 200 mg six-hourly intravenously, heparin 1000 IU/h, and intravenous dipyridamole 100 mg with no clinical or haematological improvement. He became comatose shortly after arrival and died two days after admission. Permission for necropsy was refused.

Methods and results

Plasma samples were collected from the two patients before treatment and from 22 healthy male and female laboratory staff and stored at -30°C until assayed. Samples were buffered to pH 4.0 and prostaglandins extracted with ethyl acetate. Extracts were measured by radioimmunoassay for 6-oxo-PGF_{1α}⁴ and thromboxane B₂. The method for thromboxane B₂⁵ was modified, antibody-bound thromboxane B₂ being extracted by using donkey antirabbit IgG precipitating serum (Scottish Antibody Production Unit). The mean recovery from the extraction procedure, established with ³H-6-oxo-PGF_{1α} and ³H-thromboxane B₂ (New England Nuclear) as tracers, was $95 \pm \text{SD } 3\%$ and $94 \pm 1\%$ ($n=5$) for 6-oxo-PGF_{1α} and thromboxane B₂, respectively. Interassay and intra-assay coefficients of variation were $2.0 \pm 0.1\%$ and $3.1 \pm 0.2\%$, respectively, for 6-oxo-PGF_{1α}, and 10.7% and 12.1% , respectively, for thromboxane B₂. The table shows the values recorded.

Comment

Occasional remissions have been reported in thrombotic thrombocytopenic purpura with various forms of treatment,⁶ but our two patients failed to respond to several forms of treatment, including an infusion of PGI₂ in case 1. The plasma concentrations of 6-oxo-PGF_{1α} and thromboxane B₂ were

Plasma concentrations of 6-oxo-PGF_{1α} and thromboxane B₂ in cases 1 and 2 and 22 healthy subjects

	Case 1	Case 2	Healthy subjects	
			Range	Mean \pm SD
6-oxo-PGF _{1α} (μg/l)	0.83	0.80	0.16–0.83	0.50 \pm 0.17
Thromboxane B ₂ (μg/l)	5.90	4.10	0.15–13.80	2.62*

*Geometric mean of skewed distribution.

normal in both patients, implying that amounts of the circulating precursor compounds PGI₂ and thromboxane A₂ were also normal. In these two patients we therefore did not find the deficiency of PGI₂ that has been suggested as a factor in the pathogenesis of thrombotic thrombocytopenic purpura, though we cannot comment on the rate of PGI₂ degradation. We conclude that 6-oxo-PGF_{1α} is detectable and may be in normal concentration in thrombotic thrombocytopenic purpura and that this condition need not be associated with a high concentration of thromboxane A₂.

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A relation between high-density-lipoprotein cholesterol and bile cholesterol saturation

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Abstract

The association of cholesterol gall stones with coronary artery disease is controversial. To investigate this possible relation at the biochemical level, bile cholesterol saturation and the plasma concentrations of triglycerides, total cholesterol, and high-density-lipoprotein cholesterol (HDL cholesterol) were measured in 25 healthy, middle-aged women. Bile cholesterol saturation index was negatively correlated with HDL cholesterol. It was positively correlated with plasma triglycerides and with total cholesterol minus HDL cholesterol.

These findings provide a biochemical basis for a positive association in women between cholesterol gall stones and coronary artery disease.

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Introduction

The association of cholesterol gall stones with coronary artery disease is controversial. Some early necropsy studies showed an increased prevalence of gall stones in patients with severe coronary artery disease¹ and in patients who had died of myocardial infarction,^{2,3} whereas another study did not find any definite relation.⁴ Later epidemiological studies were also conflicting, showing, in women, both positive⁵ and negative⁶ associations.

A way to investigate the association may be to look for correlations between plasma and biliary lipid concentrations. Conceivably, a low concentration of high-density-lipoprotein cholesterol (HDL cholesterol) might underlie and explain a positive association between ischaemic heart disease and cholesterol gall stones. Cholesterol is kept in solution in bile by the action of bile acids and phospholipids. If the cholesterol excreted into bile exceeds this solubilising capacity, bile becomes supersaturated with cholesterol and apt to precipitate gall stones.⁷ HDL cholesterol is an important protective factor against coronary artery disease.⁸⁻¹¹ The cholesterol taken up by high-density lipoprotein is believed to be excreted in bile.^{12,13}

but it is not known whether it is excreted as such or after conversion to bile acids.

We have examined some relations between bile cholesterol saturation, on the one hand, and the plasma concentration of HDL cholesterol and other plasma lipids, on the other.

Subjects and methods

Thirty healthy women volunteered for the study. To minimise possible variation in plasma and biliary lipid concentrations due to age and body fatness, we selected only women aged 40-45 years and who were 100-110% of ideal body weight. None was taking any medication, including oral contraceptives. All had normal standard liver function values. Oral cholecystography was omitted on ethical grounds, but no subject had symptoms suggestive of gall-bladder disease. Three of the women were found to have hyperlipidaemia and were excluded. Two others were excluded because their bile samples were too weak for accurate analysis (total lipid concentration less than 20 mmol/l). To avoid possible fluctuations in bile and plasma lipid concentrations related to the menstrual cycle¹⁴ samples were collected within the first five days of each woman's cycle.

Subjects abstained from drinking alcohol for one week before testing. After an overnight fast bile and venous blood were sampled simultaneously. Bile-rich duodenal fluid was collected after duodenal intubation and intravenous injection of cholecystokinin. This elicited a prompt flow of concentrated bile in all cases. The composition of bile thus obtained reflects accurately that of gall-bladder bile.¹⁵

Total bile salt, phospholipid, and biliary cholesterol concentrations were measured.¹⁶ Individual bile acid composition was determined by gas-liquid chromatography. Bile cholesterol saturation index was calculated by the method of Thomas and Hofmann¹⁷ using the criteria of Hegardt and Dam.¹⁸ Plasma cholesterol¹⁹ and triglyceride²⁰ concentrations were measured by routine autoanalyser methods. HDL cholesterol was measured by a manual cholesterol oxidase method (Boehringer Mannheim GmbH 236691) after precipitation of the other lipoproteins with heparin and manganese chloride.²¹ The statistical significance of correlation coefficients was determined by Student's *t* test.

Results

Mean bile cholesterol saturation index was $1.09 \pm \text{SEM } 0.15$, and 18 of the 25 women had supersaturated bile. Mean plasma lipid values were: plasma triglycerides 0.73 ± 0.21 mmol/l (64.6 ± 18.6 mg/100 ml), total plasma cholesterol 4.98 ± 0.90 mmol/l (192.3 ± 34.7 mg/100 ml), and HDL cholesterol 1.58 ± 0.25 mmol/l (61.0 ± 9.7 mg/100 ml).

Bile saturation index was positively correlated with plasma triglyceride concentration ($r=0.471$; $p<0.02$; fig 1) but not with total plasma cholesterol concentration ($r=0.319$; NS). Saturation index was negatively correlated with HDL cholesterol ($r=-0.509$; $p<0.01$; fig 2). There was no significant correlation between HDL cholesterol and plasma triglycerides ($r=-0.346$). The association of HDL cholesterol and plasma triglycerides with bile cholesterol

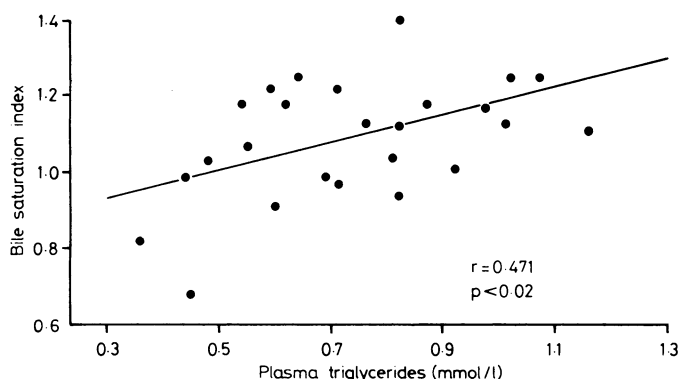


FIG 1—Correlation of bile cholesterol saturation index and plasma triglyceride concentration in 25 normolipidaemic women.

Conversion: SI to traditional units—Triglycerides: 1 mmol/l \approx 88.5 mg/100 ml.

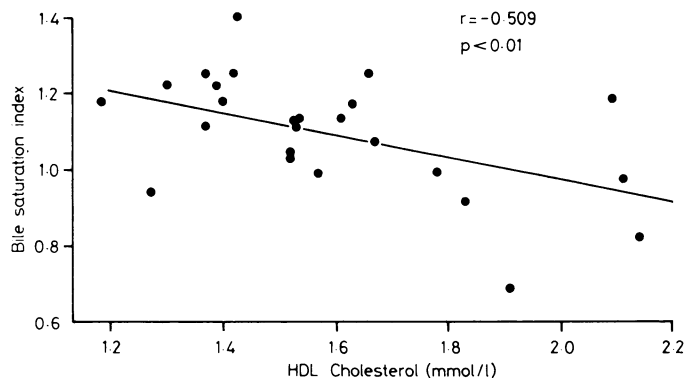


FIG 2—Correlation of bile cholesterol saturation index and HDL cholesterol concentration in 25 normolipidaemic women.

Conversion: SI to traditional units—HDL cholesterol: 1 mmol/l \approx 38.6 mg/100 ml.

saturation was shown by multivariate analysis to be partially independent.

HDL cholesterol was subtracted from total cholesterol to give a measure of the cholesterol contained in low-density lipoprotein and very-low-density lipoprotein. This value showed a significant positive correlation with bile saturation index ($r=0.597$; $p<0.005$; fig 3). No significant correlations were found between the proportions of the individual bile acids with the various plasma lipids.

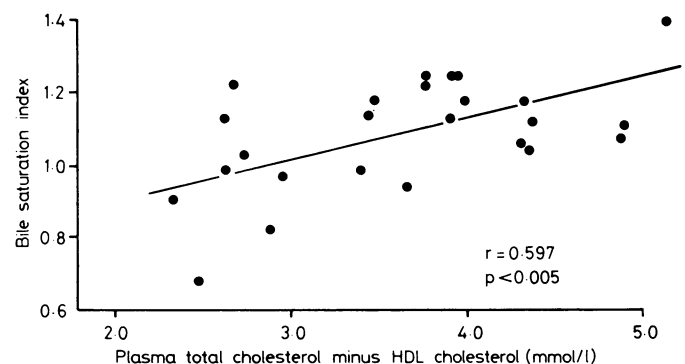


FIG 3—Correlation of bile cholesterol saturation index and plasma concentration of total cholesterol minus HDL cholesterol in 25 normolipidaemic women.

Conversion: SI to traditional units—Cholesterol and HDL cholesterol: 1 mmol/l \approx 38.6 mg/100 ml.

Discussion

This study shows that in healthy middle-aged women bile cholesterol saturation is inversely correlated with the plasma HDL cholesterol concentration. This is consistent with a recent report that women with a history of gall-bladder disease have a reduced concentration of HDL cholesterol.²²

We have also shown that a relation exists between bile cholesterol saturation and plasma triglycerides, not only in hypertriglyceridaemia²³ but also when the triglyceride concentration is normal. Plasma triglycerides are transported by very-low-density lipoprotein, which is catabolised with the removal of triglyceride to low-density lipoprotein^{24 25}; this lipoprotein carries most of the plasma cholesterol, the remainder being transported by high-density lipoprotein. High-density lipoprotein is thought to facilitate the egress of cholesterol from atheromatous plaques^{26 27} and inhibit tissue uptake of cholesterol from low-density lipoprotein.²⁸ If the cholesterol taken up and transported to the liver by high-density lipoprotein is secreted into bile wholly or predominantly as cholesterol, then high concentrations of high-density lipoprotein might be envisaged as increasing the risk of gall stones. Conversely, if HDL

cholesterol is converted to bile acids, then a high concentration of the lipoprotein, in addition to being antiatherogenic, might also protect against gall stones.

It has been suggested that bile acids and biliary cholesterol are derived from separate hepatic pools of cholesterol²⁹ and that these pools are derived mainly from lipoproteins, rather than newly synthesised cholesterol.³⁰ High-density lipoprotein has been proposed as the main lipoprotein contributor to both these pools^{12,13}—but on the basis of experiments in a single patient. The inverse correlation of HDL cholesterol and bile cholesterol saturation throws doubt on the suggestion¹² that cholesterol from high-density lipoprotein is an important source of biliary cholesterol. Low-density lipoprotein,³¹ like high-density lipoprotein,²⁶ may have an important role in returning cholesterol to the liver. The positive correlation of bile cholesterol saturation with total plasma cholesterol minus HDL cholesterol (largely low-density lipoprotein cholesterol) might indicate that biliary cholesterol is derived principally from low-density lipoprotein.

As to whether cholesterol derived from high-density lipoprotein is converted to bile acids,¹³ the inverse correlation of bile cholesterol saturation and HDL cholesterol would be compatible with this idea. From our data, however, we cannot comment on whether HDL cholesterol is the sole or even the main contributor of the cholesterol utilised for bile acid synthesis.

The data suggest that measures which alter HDL cholesterol concentration, either beneficially—for example, exercise,³²⁻³⁴ moderate alcohol consumption^{34,35}—or detrimentally—for example, cigarette smoking³⁴—may have similar, as yet unreported effects on the cholesterol saturation of bile.

In conclusion, our findings disclose a relation in women between a major risk factor for ischaemic heart disease and the metabolic basis of cholesterol cholelithiasis. The data provide a biochemical basis for a positive association between gall stones and coronary artery disease.

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PERSONS not skilled in anatomy ought never to bleed in a vein that lies over an artery or a tendon, if they can avoid it. The former may easily be known from its pulsation or beating, and the latter from its feeling hard or tight like a whip cord under the finger.

IT was formerly a rule, even among those who had the character of being regular practitioners, to bleed their patients in certain diseases till they fainted. Surely a more ridiculous rule could not be proposed. One person will faint at the very sight of a lancet, while another will lose almost the whole blood of his body before he faints. swooning depends more upon the state of the mind than of the body; besides, it may often be occasioned or prevented by the manner in which the operation is performed.

(Buchan's *Domestic Medicine*, 1786.)