Secretion of biliary lipids in young Chilean women with cholesterol gallstones

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SUMMARY The early appearance of cholesterol gallstones is very common in Chile. To elucidate the mechanisms involved in this phenomenon, the size of the bile acid pool and the secretion of biliary lipids were studied in two groups of young women with normal weights and radiologically functioning gallbladders: nine with cholesterol gallstones and 14 healthy volunteers. The bile acid pool was similar in control and gallstone patients. The secretory rates of bile acids and phospholipids were comparable and significantly correlated in both groups. On the other hand, cholesterol output was higher in gallstone patients. In controls there was a significant correlation between the output of bile acids and cholesterol, but no correlation was found in the gallstone group. This study suggests that cholesterol hypersecretion into the bile is a major factor in the pathogenesis of cholesterol gallstone disease in young Chilean women with normal weights.

Although it is widely held that the hepatic secretion of bile supersaturated with cholesterol is required to develop cholesterol gallstones, this phenomenon occurs intermittently in healthy individuals without gallstones (Northfield and Hofman, 1975). Bile may become supersaturated with cholesterol as a result of excess cholesterol and/or low bile acid and lecithin secretion.

Studies of the secretion of biliary lipids in cholesterol gallstone disease have yielded different results. While some workers have found no differences in the output of biliary lipids between gallstone and control subjects (Northfield and Hofmann, 1975) others have reported high cholesterol outputs in obese Caucasians with gallstones (Grundy *et al.*, 1974; Shaffer and Small, 1977). In contrast, low secretory rates of bile acids and lecithins have been found in gallstone patients with normal weights (Shaffer and Small, 1977). Furthermore, both mechanisms seem to coexist in American Indians of the Southwestern United States (Grundy *et al.*, 1972).

The early appearance of cholesterol gallstones is a remarkable feature of this disease in Chilean women. Studies in a necropsy series (Marinovic *et al.*, 1972)

and in a living population (Covarrubias *et al.*, 1976) have shown that, in Chile, 5–8% of adolescent girls and 25–30% of young adult women have cholelithiasis or have already been operated on. Our aim was to study the secretion of biliary lipids and bile acid pool size in young, non-obese women with cholesterol gallstones and in matched healthy controls to understand the pathogenesis of the disease in the Chilean population. This study is particularly relevant, as Chile may have the highest frequency of cholesterol gallstones published in the world (Marinovic *et al.*, 1972).

Methods

SUBJECTS

Nine women with radiolucent stones in a functioning gallbladder were compared with 14 healthy controls with a normal cholecystogram matched for age and weight (Table 1). None of them was obese or had the phenotypical characteristics of pure Chilean Indians. All the subjects volunteered for the studies. Informed, written consent was obtained in accordance with the standards required by the Research Committee of the Medical School, Universidad Católica de Chile. Eight of the nine gallstone patients had cholecystectomies performed after the study, and their stones were found to contain 60–98% of cholesterol.

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Table 1 *Ages, heights, and weights of two groups studied*

Group	No.	Age (yr)	Height (cm)	Weight (kg)	% Ideal weight
Controls	14	20.6 ± 0.6	161 ± 1.9	51.6 ± 1.4	90.1 ± 2.1
Gallstone patients	9	21.2 ± 1.4	158 ± 1.9	53.0 ± 2.5	95.3 ± 3.8

Patients and healthy volunteers were admitted to the hospital for eight days, and given a standard diet (30 kCal/kg body weight/day, 1g protein/kg body weight/day, and 30% of the calories as lipids). On the last day, the hourly secretion rates of biliary lipids were measured by a marker perfusion technique (Grundy and Metzger, 1972) and the bile acid pool size was determined by isotope dilution (Duane *et al.*, 1975). Briefly, the subjects were intubated the night before with a triple-lumened polyvinyl tube, and 2 Ci¹⁴C-carbonylcholic acid was infused into the stomach. Next morning the infusing orifice and the proximal collecting port were adjusted by x-rays to the second portion of the duodenum. The distal collecting orifice was situated 10cm further along. Thirty millilitres of a solution of aminoacids were infused and fasting gallbladder bile was collected. A sample of 1ml was saved and the rest returned to the duodenum. Then a standard meal containing ³Hβ sitosterol as a non-absorbable marker was constantly infused into the duodenum to obtain tonic contraction of the gallbladder and to provide a diet of the same composition and caloric value as the diet of the previous days. After an equilibrium period of four hours, duodenal content was continuously aspirated from the proximal and distal collecting orifices during the following steady state period of eight hours.

The samples of gallbladder bile were assayed for bile acids (Talalay, 1960) and ¹⁴C radioactivity. The ratio of ¹⁴C to total bile acids ('specific activity') was determined in triplicate and the bile acid pool was estimated by dividing the dose of radioactivity given by the mean 'specific activity'.

The duodenal samples aspirated from the distal orifice were assayed for cholesterol (Abell *et al.*, 1952) and ³Hβ sitosterol. The concentrations of bile acids, phospholipids (Baginski *et al.*, 1967), and cholesterol were determined in the duodenal samples aspirated from the proximal collecting port. With these data, hourly biliary lipid outputs were calculated as described (Grundy and Metzger, 1972). The infused meal contained a small amount of cholesterol, and was virtually free of phospho-

lipids. The values of cholesterol withdrawn from the duodenum were corrected from the exogenous cholesterol.

Data are expressed as mean ± SEM. Their statistical significance was evaluated by Student's *t* test.

Results

BILE ACID POOL

The bile acid pool size was similar in controls and in gallstone patients (1.78 ± 0.14g vs 2.30 ± 0.34g, NS). Furthermore, its recycling rate (calculated daily output of bile acids divided by the pool size) was also comparable in both groups: 10.8 ± 1.4 and 11.7 ± 1.9 times per day respectively. Thus, under these experimental conditions, the speed of the entero-hepatic circulation of bile acids is similar in healthy controls and in patients with cholelithiasis.

OUTPUTS OF BILIARY LIPIDS

The hourly outputs of bile acids and phospholipids were comparable in both groups, but the cholesterol secretion rates were significantly higher in gallstone patients than in healthy controls (Table 2). A positive and significant correlation was found between the outputs of bile acids and phospholipids both in normal volunteers and in gallstone patients. The slopes of the regression lines were also similar in both groups (Fig. 1). Quite different results were found in the correlation study of bile acid and cholesterol secretion (Fig. 2). While this correlation was clear and significant in healthy controls, we did not find a significant correlation between these two parameters in the gallstone patients.

LIPID COMPOSITION OF HEPATIC BILE

The lithogenic indexes of hepatic bile were calculated (Thomas and Hofmann, 1973) from the molar percentages of biliary lipids found in the proximal duodenal samples, using the cholesterol saturation limits described in human bile by Holzbach *et al.* (1973). The liver secreted non-saturated bile throughout the experiments both in healthy women and in gallstone patients. Although the mean lithogenic index was higher in the latter (0.80 ± 0.07 vs 0.70 ±

Table 2 *Hourly outputs of biliary lipids*

Group	No.	Bile acids (mg/h)	Phospholipids (mg/h)	Cholesterol (mg/h)
Controls	14	775 ± 52	322 ± 31	33.6 ± 2.1
Gallstone patients	9	949 ± 135	399 ± 54	44.6 ± 1.3
P		NS	NS	<0.005

Fig. 1 Correlation between bile salt and phospholipid secretions in control women and gallstone patients Each point represents the mean hourly output of these two lipids in a single study.

Fig. 2 Correlation between bile salt and cholesterol secretions in control women and gallstone patients Each point represents the mean hourly output of these lipids in a single study.

0.05) this difference does not reach statistical significance.

Discussion

The present studies were performed to identify potential pathogenic factors of cholesterol gallstone disease in non-obese young Chilean women. We found that the size of the bile acid pool was comparable in controls and gallstone patients. Similarly, the outputs of bile acids and phospholipids, under continuous stimulation of the enterohepatic circulation, were comparable and significantly correlated in both groups. The absolute values of bile acid and phospholipid secretion were in the range of those previously reported for the Caucasian women without gallstones (Grundy *et al.*, 1974). It therefore appears that neither a deficiency in bile acid secretion nor a failure in the synthesis or canalicular secretion of phospholipids should play an important role in the pathogenesis of this disease in Chilean women. On the other hand, the output of cholesterol was

significantly higher in our gallstone patients. In fact, their cholesterol secretion rates were within the range observed in obese Caucasian women with gallstones (Grundy *et al.*, 1974) and in American Indian women with cholelithiasis (Grundy *et al.*, 1972). It must be stressed, however, that our patients were not obese and, when compared with the American Indians, their secretory rates of bile acids and phospholipids were remarkably higher. It therefore appears that bile lithogenicity in young Chilean women with cholesterol gallstones is mainly explained by the hypersecretion of biliary cholesterol.

While cholesterol secretion was significantly correlated with bile acid secretion in the control subjects, no correlation was found in our gallstone group. As it has been reported that, within certain limits, biliary cholesterol secretion is dependent on bile acid secretion (Scherstén *et al.*, 1971; Northfield and Hofmann, 1975), it may be possible that in our gallstone patients a major fraction of biliary cholesterol was secreted independently of biliary bile acids. It is also worthwhile to stress that, under continuous enterohepatic circulation of the bile salts, our gallstone patients secreted a nonsaturated hepatic bile. Therefore, the supersaturation of bile observed after the overnight fast (Metzger *et al.*, 1973) or because of insufficient stimulation of the gallbladder contraction, probably plays an important role in the pathogenesis of the disease in Chilean women.

The possibility that a racial factor is a major determinant in the results of this study cannot be ruled out because the admixture of American Indian blood is common in our country. Nevertheless, it must be stressed that none of the volunteers studied had the phenotypical characteristics of pure Chilean Indians. Environmental factors may therefore also be important in cholesterol gallstone formation in the Chilean population.

This study was supported by Grant 109/76 from the Dirección de Investigación, Universidad Católica de Chile, and by Grant 40/78R, Servicio de Desarrollo Científico, Universidad de Chile. The authors are grateful to the nutritionists and nurses of the Metabolic Units of both hospitals for their expert technical assistance. They also wish to thank Dr. Scott M. Grundy for his generous help.

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